THE DEVELOPMENT OF A SYSTEM TO MEASURE THE EFFECTS OF PLANTAR FOOT PRESSURE ON THE MICROCIRCULATION OF THE FOOT

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ABSTRACT

An investigation into the effects of plantar foot pressure on the microcirculation of healthy subjects and patients with Rheumatoid Arthritis was carried out. In the light of no equipment available to carry out this study a new system was developed. A shoe device was built with a combined pressure/skin blood flow transducer embedded in a three-tier piston mechanism in the heel so that plantar foot pressure could be applied/removed and quantified. The skin blood flow transducer made contact with the skin and was able to collect data about the microcirculatory state of the skin.

The first system developed consisted of the laser Doppler Fluxmeter (Moor Instruments Ltd., UK) used to collect skin blood flow information and incorporating a strain gauge (Kyowa Electronic Instruments Co. Ltd., Japan) to quantify plantar foot pressure applied to the centre of the heel. This system was visually/sound synchronised and due to the time delay error it was modified. For the final system developed, the strain gauge was replaced with a custom-made Novel capacitative transducer (Novel, Germany) to quantify pressure. This allowed for the pressure system to be electronically synchronised in real time with the laser Doppler fluxmeter using an electronic synchronisation box. A number of studies were carried out to validate the systems.

The developed systems were used to: (a) investigate the effects of the venoarteriolar response in healthy subjects with regards to the effects of

plantar foot pressure on skin blood flow. The study concluded that subject positioning (that is, supine or semi-weight bearing) has an effect on how the microcirculation of the skin reacts to applied pressure. Thus, studies investigating the effects of external pressure on skin blood flow must have their subjects in a position that is related to what is being studied; (b) investigate the effects of plantar foot pressure on skin blood flow in patients with Rheumatoid Arthritis. A healthy control group was compared with a cohort of patients with Rheumatoid Arthritis with no evidence of vasculitis. The study concluded that there were no significant differences between both groups.

A number of articles have been published from this thesis (see Appendix 14).

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CHAPTER 1

INTRODUCTION

1. CHAPTER 1: Introduction

1.1 Background to Research

Rheumatoid arthritis is a relentless and progressive disease affecting 0.5 to 1.5% of the population (Lau *et al.,* 1996; Michelson *et al.,* 1994; Hochberg, 1981).

> *"Of all the connective tissue disorders, rheumatoid arthritis is not only the most common but the most likely to lead to severe and often irreparable deformity of the small joints of hands and feet. The extra-articular manifestations of the disease may exacerbate joint problems and result in instability, severe limitation of mobility and pain."* (Braid, 1996)

A study found that 50% of patients with rheumatoid arthritis for less than ten years had significant foot problems, increasing to 75% for those with rheumatoid arthritis for more than twenty years (Michelson *et al.,* 1994). As the disease progresses there is joint damage and loss of functional ability, which together with pain, results in disability with approximately 50% of those affected having to retire from work within ten years of the disease onset (Braid, 1996; Platto *et al.,* 1991; Yelin *et al.*, 1987; Makisara & Makisara, 1982). In addition to joint damage vasculitis affects approximately 19.8/million of patients with rheumatoid arthritis annually, with the incidence peaking in the 65-74 years age group (Watts *et al.,* 1995). Apart from the increased morbidity, there is an increased mortality rate amongst patients with rheumatoid arthritis when compared to the general population (McEntegart *et al.,* 2001; Hochberg & Spector, 1990). Since patients with rheumatoid arthritis are at high risk of severe foot deformity affecting plantar foot pressure distribution and blood vessel disease they are an ideal group to study the effects of plantar foot pressure on skin blood flow.

Rheumatoid arthritis often commences in the foot, particularly at the metatarsophalangeal joints with synovitis and atrophy of the fat pad (Resnick *et al.*, 1999; West & Woodburn, 1995). It has been reported that in conditions like rheumatoid arthritis the shock absorbing mechanism of the

fat pad is diminished (Rome, 1998). The presenting complaint is usually forefoot deformity, however, as the disease progresses rearfoot deformities commence as a result of excessive pronatory forces on a weakened and inflamed subtalar joint decreasing the stability of the foot, leading to deterioration of the knee and increasing disability (Braid, 1996; Saltzman *et al., 1995;* Keenan *et al.,* 1991). With the disease progression, anterior shift of the fat pad resulting from failure of instrinsic muscles, foot instability and clawing of toes, leads to prominent metatarsal heads and high plantar foot pressure (Wiener-Ogilvie, 1999). Bursa may develop over these prominent joints, which themselves may become inflamed (Braid, 1996). In some cases rheumatoid nodules may form under some of the affected joints (Braid, 1996). These pathogenic plantar foot pressures are increased further by a change in the ratio of saturated to unsaturated fat reducing the cushioning effect of the fat pad in patients with rheumatoid arthritis (Resnick *et al.,* 1999). In some cases pathogenic plantar foot pressures, especially where neuropathy exists leads to soft tissue breakdown and ulceration (Braid, 1996; Proano *et al.,* 1992; Livingston, 1992). Injury to the soft tissues may occur as a result of a constant pressure maintained over several hours, high pressure held over a short period and/or by a repetitive moderate pressure (Proano *et al.,* 1992). Recent research in rheumatoid arthritis has correlated plantar foot pressures with levels of pain and heel position, and prescriptions of orthosis with reductions in pain (Li *et al.,* 2000; Woodburn *et al.*, 2000; Hodge *et al.*, 1999; MacSween *et al.*, 1999; Woodburn & Halliwell, 1996[a]).

Vasculitis, which usually occurs in severe rheumatoid arthritis, is one of the most serious extra-articular manifestations and may be life-threatening causing inflammation of blood vessels leading to damage of many organs, including the skin (Koutantji *et al.,* 2000; Braid, 1996). Vasculitic infarcted tissue commonly appears as small brown/black spots on apices and periungual areas (Braid, 1996). A study by Edwards (1980) found a close correlation between sites of blanching (i.e. pressures sites) in the hand and sites of vasculitis in patients with rheumatoid arthritis and suggested a possible correlation between vascular compression and blood stasis in the

development of rheumatoid vascular disease. These vasculitic lesions are painful and at risk of causing tissue breakdown and ulceration (Braid, 1996).

In medical practice most of the vascular assessments involves determining segmental pressure and flow velocities, however physiologically, adequate cellular metabolism is most dependant on its microcirculatory haemodynamics rather than its macrocirculatory state, thus macrocirculatory tests may in some cases only estimate the level of tissue ischaemia (Pabst *et al.,* 1985). In diabetes mellitus autonomic neuropathy leads to arteriovenous shunting, which causes a very rapid flow of blood, leading to distension of veins and high oxygen concentrations in the venous blood and severely restricting the amount of blood reaching the skin's nutritive capillaries and side channels (Santos *et al.,* 2000; Ward *et al.,* 1983; Boulton *et al.,* 1982). In this example although the foot is warm with bounding pulses the skin's nutritive blood supply is not adequate, starving the foot of oxygen and nutrients and at risk of soft tissue breakdown and ulceration, thus directly assessing skin blood flow may in some cases be more important than just assessing for macrovascular disease (Santos *et al.,* 2000; Ward *et al.,* 1983). In addition, assessing endothelial dysfunction within the microcirculation may be used as a marker of cardiovascular disease (Sessa, 2004; Wierzbicki *et al.,* 2004; Behrendt *et al.,* 2002; Vita *et al.,* 2002; Fichtlscherer *et al.,* 2000; Hingorani *et al.,* 2000; Schachinger *et al.,* 2000; Suwaidi *et al.,* 2000; Luscher *et al.,* 1997). In the past few years measurements of endothelial dysfunction in the forearm and the fingertip has been associated with cardiovascular disease (www.endothelix.com, 2005); Wierzbicki *et al.,* 2004; Fichtlscherer *et al.,* 2000).

The skin is one of the largest organs in the body, is part of the integumentary system and its microcirculation plays a vital part in the regulation of an individual's metabolic, haemodynamic and thermal state (Seely *et al.,* 2000[a]; Tortora *et al.,* 1993[a]; Stern, 1975). The skin's physiological state is complex in nature with both central and local control and changes constantly over short and long periods (for example, its reacts quickly to external stimuli or reacts and deteriorates slowly with slowly

progressing vascular disease) (Stern, 1975). In youth, the epidermal cells are orientated around their source of nourishment with the active elongated basal cells pushing the epidermis inward and forming ridges along the epidermal/dermal junction. During the aging process atrophy of the epidermis and its cutaneous supply occurs with the skin appearing thin and the epidermal/dermal border appearing "flat" with many basal cells showing little activity and sense of orientation. In addition, hypertrophy of the epidermis appears to be closely correlated with hypertrophy of the papillary vessels, and atrophy of the epidermis with atrophy of these papillary vessels (Livingston, 1992). Thus, any disease affecting the cutaneous nutritive blood supply to the skin will cause atrophy of the skin and reduce its stress relieving mechanisms. This will result in the skin being less able to tolerate excessive stresses and strains. In rheumatoid arthritis, as the disease progresses, the skin takes a similar thin, flat appearance to that in old age suggesting some form of microvascular disease and less prone to recover from pressure ischaemia and at risk of cutaneous cellular death and ulceration. Thus, assessing skin blood flow is a good method of determining the presence of vascular disease or injury. In rheumatoid arthritis, sites where peripheral blood flow is poor may deteriorate and cause ischaemic ulcers (Braid, 1996).

> *"One of the prime mechanical factors for tissue breakdown of the skin, leading to pressure sore formation, is beyond question the external pressure over a bony prominence."* (Schubert *et al.,* 1994).

When external pressure is applied to soft tissue, its effects are complex and depend on the magnitude and duration of the applied pressure and the anatomical site (Abu-Own *et al.,* 1995). In comparison to other sites of the human body, the skin on the plantar aspect of the foot spends most time in direct contact with the external environment (Saltman *et al.,* 1995). To protect the foot from injury, through evolution, the foot has developed three main protective mechanisms: firstly, the skin is thicker in the plantar aspect of the foot to protect against mechanical injury; secondly, sole to ground frictional properties are controlled by the development of deep epidermal-

dermal ridges to anchor the epidermis and prevent injury; and finally, a rich network of afferent nerve endings provides continuous feedback of the mechanical environment (Saltman *et al.,* 1995). Where the nutritive blood supply to the skin is affected the epidermal-dermal ridges appear flat with the skin losing some of its protective properties (Livingston, 1992). During the gait cycle there is repetitive loading of the calcaneal soft tissues with the intensity of these stresses increasing and related to the vigour of gait or activity (Perry, 1983). These vertical ground reaction forces will exceed body weight (Saltman *et al.,* 1995). The effects of plantar foot pressure on skin with a defective nutritive cutaneous blood supply increases the chances of cellular death and ulceration to occur, especially in high-risk patients suffering from rheumatoid arthritis or diabetes mellitus (Braid, 1996; Proano *et al.,* 1992; Livingston, 1992).

To assess the effects of plantar foot pressure on skin blood flow, a non-invasive, reliable, rapid and a system that interferes minimally with the many local and central microcirculatory responses is necessary. Over 25 years ago, Stern (1975) described the *in vivo* evaluation of skin blood flow using a monochromatic beam of coherent light. At the time measurement of skin blood flow depended on direct observation, radioisotope, thermal or plethysmography techniques. These techniques were slow, cumbersome and provided unreliable readings. The laser Doppler fluxmeter was developed to measure non-invasively the microcirculation using the Doppler principle that light reflected from moving objects (mainly red blood cells in the vascular vessels of soft tissue) would cause a shift in the laser's light frequency related to the speed of moving erythrocytes (Raamat *et al.,* 2001; Abbot *et al.,* 1993). Other studies using the laser Doppler imager and laser Doppler fluxmetry have reported that heel blood perfusion, especially in the central heel area, is rapidly and significantly reduced on pressure loading causing pressure-induced ischaemia and may lead to pressure-induced ulceration (Mayrovitz *et al.,* 1999; Mayrovitz *et al.,* 1998; Mayrovitz *et al.,* 1997). Using laser Doppler flowmetry Fromy *et al.* (1997), found that in healthy young subjects, when an external uniform pressure as low as 10 mmHg (1.33 kPa) is applied to the skin, significant impairments in both

arterial inflow of the lower limb and microcirculation of the forefoot occurs. Proano *et al.* (1992) found that plantar foot pressure above 3 Ncm^{-2} (300 kPa) is sufficient to arrest the nutritive blood flow to the skin in the foot. The areas on the foot exceeding this pressure are the hallux, the plantar metatarsal heads and the heel (Proano *et al.,* 1992). In diabetics, Newrick *et al.* (1988) found that the highest static plantar foot pressure occurred under a metatarsal. In addition, when measuring skin blood flow under the metatarsal with the highest pressure there were significant differences between neuropathic and non-neuropathic patients and healthy subjects. In rheumatoid arthritis, Li *et al.* (2000) found the plantar foot peak pressure[1] distribution to be 7.59 g/cm^2/body weight [2] in the rearfoot, 5.80 g/cm^2/body weight in the midfoot and 5.69 g/cm^2/body weight in the forefoot. In comparison, Hodge *et al.*, 1999 reported higher pressures in the forefoot that in other parts of the foot. However, the number of studies investigating the effects of external pressure on the microcirculation of the skin are few and none have investigated the effects of plantar foot pressure on skin blood flow with the subjects in a semi-weight bearing position (Santos *et al.,* 2002; Cobb *et al.,* 2001; Mayrovitz *et al.,* 1998; Mayrovitz *et al.,* 1997; Meinders *et al.,* 1996; Abu-Own *et al.,* 1995; Abu-Own *et al.,* 1993; Proano *et al.,* 1992). Furthermore, there are no studies investigating the effects of plantar foot pressure on skin blood flow in patients with rheumatoid arthritis.

No commercially available equipment exists that is capable of measuring the effects of plantar foot pressure on skin blow flow and all the research in this field has been done with experimental systems. Previous studies have investigated the validity and reliability of the laser Doppler flowmeter, including measuring skin blood flow under progressive calibrated pressure, and have produced guidelines and protocols (Fromy *et al.,* 2000; Michelson *et al.*, 1996; Serup, 1994; Latino *et al.*, 1994; Vongsavan *et al.*, 1993[a]; Vongsavan *et al.*, 1993[b]; Pietila *et al.,* 1988; Saumet *et al.,* 1988). The laser Doppler fluxmeter will be used to measure the effects of externally applied quantifiable pressure on skin blood flow in the centre of the plantar aspect

[1] Maximum pressure recorded in the sole of the foot by transducers during ambulation.
[2] Pressure in g/cm^2 divided by subjects own body weight.

of the heel. In addition, the in-shoe Pedar plantar foot pressure system has been shown to be a valid and reliable tool to measure plantar foot pressure with high coefficient of repeatability values above 0.75 (dependant on site tested and number of steps), thus the system will be adapted for this task in this thesis (Finch, 1999; Hodge *et al.,* 1999; Kernozek *et al.,* 1996).

The evidence suggests that the progressive nature of rheumatoid arthritis leads to deformity and a reduction in the cushioning properties of the sole of the foot producing higher than 'normal' plantar foot pressure. The high plantar foot pressure may be responsible for eliciting foot pain (maybe associated with decreased or arrested skin blood flow), and reducing high plantar foot pressures with orthosis seems to reduce pain levels but the mechanism associated with pain reduction apart from the obvious decrease in plantar foot pressures is not known. In addition, the studies on skin blood flow suggest that when pressure is applied to the skin, cutaneous blood flow may be impaired and in some cases lead to pressure-induced ischaemia, soft tissue damage and ulceration. However, an extensive literature search did not reveal any evidence regarding the effects of plantar foot pressures on skin blood flow in rheumatoid arthritis subjects in comparison to controls, or the effects of pressure reducing strategies using orthosis on the microcirculation and possible benefits as a clinical intervention by reducing pressure-ischaemia. Furthermore, no commercially available equipment exists that is capable of measuring the effects of quantifiable plantar foot pressure on skin blood flow.

In this project, a system to measure the effects of quantifiable plantar foot pressure on skin blood flow in the plantar aspect of the heel was developed. This was followed by studying the effects of static plantar foot pressure on skin blood flow in the sole of the foot of patients with rheumatoid arthritis.

Insoles are prescribed regularly or commonly for patients with pain or neuropathic feet to reduce plantar foot pressure, how this affects the microcirculation in "high risk" patients or the mechanism that causes cellular death, soft tissue breakdown and ulceration is yet to be understood. This

study provides a better understanding of the effects of plantar foot pressure on skin blood flow in rheumatoid arthritis.

1.2 Research aim

The aim of the study is to develop and validate an experimental system to measure the effects of plantar foot pressure on skin blood flow; and investigate, contribute, and strengthen current knowledge of the effects of plantar foot pressure on the microcirculation of the foot in patients with rheumatoid arthritis.

1.3 Research objectives

(1) To develop and validate a system to measure the effects of plantar foot pressure on skin blood flow with the subject in a supine and semi-weight bearing position[3].

(2) To investigate and strengthen current knowledge of the effects of the postural vascular response on the effects of plantar foot pressure on skin blood flow.

(3) To study and contribute to current knowledge of the effects of plantar foot pressure on skin blood flow of patients with rheumatoid arthritis.

1.4 Justification for the research

Investigating the effects of plantar foot pressure on skin blood flow is vital if we are to increase our knowledge of the complex and interrelated mechanisms that contribute to the development of soft tissue breakdown and ulceration of the foot. However although plantar foot ulcers are not a common feature in rheumatoid arthritis, unless vascular disease is present, this group of patients does suffer from a high rate of foot deformity that leads to high plantar foot pressure. Studies investigating the effects of externally applied pressure on unicellular organisms found that such pressures would have to be enormous before damage to cellular function

[3] The system was developed to measure in the two positions mentioned because the influence of the postural vascular response when measuring the effects of plantar foot pressure on skin blood flow is not known and has to be investigated to work out the optimum position for subjects to adopt in this thesis and future studies.

occurred (Cattell, 1936). Thus, the mechanisms involved in developing plantar foot pressure related soft tissue breakdown must be unrelated to cellular destruction by physical means and more related to cellular destruction by disruption of the nutritive vascular supply and causing cellular death by starvation, leading to soft tissue breakdown and ulceration.

> *"The damaging effects of pressure in higher animals and in humans must, therefore, be related to the interference with the circulatory function and the characteristics of extracellular tissues."* (Abu-Own *et al.,* 1995).

In the "at risk" rheumatoid foot, pathogenic plantar foot pressure, especially where neuropathy/vasculitis exists may result in cellular death within soft tissues and ulceration developing (Braid, 1996; Proano *et al.,* 1992; Livingston, 1992). However, before future research studies commence to investigate the benefits of plantar foot pressure reduction with orthosis in rheumatoid arthritis with the purpose of preventing soft tissue damage, we need to establish the mechanisms of cellular death and soft tissue breakdown by pathogenic plantar foot pressure. In order to establish research evidence as to the possible mechanisms that leads to mechanically induced soft tissue injury, this thesis will investigate the effects of plantar foot pressure on skin blood flow in patients with rheumatoid arthritis.

The current theses was carried out because no adequate quantitative research exists with regards to the response and/or disruption of local skin blood flow by externally applied quantifiable pressure, especially in the foot and with the subject in an upright or other position were the venoarteriolar response can be activated (e.g. semi-weight bearing). This thesis will contribute to current knowledge by describing how, using currently available equipment (that is, using the laser Doppler fluxmeter and the Pedar in-shoe plantar foot pressure system), an electronically synchronised system may be constructed, validated and used to measure the effects of quantifiable plantar foot pressure on skin blood flow with the subject in a supine or semi-weight bearing position.

Some studies have investigated the effects of external pressure on the foot with the subject in a supine position (Mayrovitz *et al.,* 1999; Mayrovitz *et al.,* 1998; Mayrovitz *et al.*, 1997; Meinders *et al.,* 1996; Abu-Own *et al.,* 1995; Abu-Own *et al.,* 1993). However, human locomotion involves an upright stance with plantar foot pressure transmitted through the skin onto deeper structures, thus understanding the subcutaneous functionality of the foot to ground interface is vital. Therefore, it may be more important to measure the physiological cutaneous blood flow responses to plantar foot pressure with subjects in an upright position if we are to understand what occurs during ambulation and its relationship to tissue viability (Santos *et al.*, 2002).

This thesis will lead the way for future studies investigating the effects of externally applied plantar foot pressure on skin blood flow, not only in rheumatoid arthritis but into other conditions where the risk of developing foot ulceration is high (for example, diabetes mellitus, Leprosy, etc.). In addition, this thesis will also encourage further work in bioengineering with the aim of improving the currently designed system to measure the effects of externally applied plantar foot pressure on skin blood flow from a semi-weight bearing to a dynamic method.

1.5 Outline of report

1.5.1 Chapter 2 (A review of methods to assess skin blood flow and plantar foot pressure)

Chapter 2 first introduces methods to assess skin blood flow concentrating on the laser Doppler fluxmeter (used in this research project to measure skin blood flow). The chapter then focuses on systems to measure plantar foot pressure and their limitations. Finally, the chapter concludes with a literature review of studies investigating the effects of external pressure on skin blood flow.

1.5.2 Chapter 3 (Anatomy, physiology and tissue viability)

Chapter 3 reviews the anatomy, physiology and optical qualities of the skin and related structures. The chapter concludes with the pathophysiology of the skin focusing on how plantar foot pressure may lead to soft tissue breakdown and ulceration.

1.5.3 Chapter 4 (Preliminary development and validation of device)

Chapter 4 concentrates on the first stage of the development process to design and validate a system to measure the effects of quantifiable plantar foot pressure on skin blood flow. The chapter concludes with recommendations to investigate whether the study in rheumatoid arthritis should be carried out with patients in a supine or semi-weight bearing position and of improvements to the system.

1.5.4 Chapter 5 (A study into the effects of postural control on plantar foot pressure on skin blood flow)

Chapter 5 studies the effects of the postural vascular response on the effects of quantifiable plantar foot pressure on skin blood flow with the subject in a supine and semi-weight bearing position and makes recommendations regarding positioning of subjects for the matched case-control study in rheumatoid arthritis.

1.5.5 Chapter 6 (Modifications and further validation of device)

Chapter 6 explains the modifications made to the system used to measure the effects of plantar foot pressure on skin blood flow. The chapter then examines the new calibration methods and validation of the equipment with recommendations to use the system to investigate the effects of quantifiable plantar foot pressure on skin blood flow in patients with rheumatoid arthritis.

1.5.6 Chapter 7 (A study investigating the effects of plantar foot pressure on skin blood flow in patients with rheumatoid arthritis)

The new validated system is used to measure the effects of plantar foot pressure on skin blood flow in patients with rheumatoid arthritis. The matched case-control study compares healthy controls with patients suffering with rheumatoid arthritis matched for age and gender.

1.5.7 Chapter 8 (General Conclusions)

The final chapter discusses the main findings of this thesis and recommendations for future research.

1.6 Definitions

Definitions adopted by researchers are not often uniform, so key and controversial terms are defined to establish the positions taken in this PhD research study with the purpose of facilitating the integration of this doctoral thesis into the wider body of literature.

Rearfoot

Rearfoot is used in this thesis to describe the anatomical parts of the body situated at the back of the foot. Some researchers refer to this term as the hindfoot.

Pedar plantar foot pressure system

The *Pedar plantar foot pressure system* is used in this thesis to describe a specially developed capacitative pressure sensor that was integrated into a three-tier piston system to measure the effects of plantar foot pressure on skin blood flow. If referring to the use of the in-shoe system, widely used by many researchers, it will be described as the "in-shoe Pedar plantar foot pressure system".

CHAPTER 2

A REVIEW OF METHODS TO ASSESS SKIN BLOOD FLOW AND PLANTAR FOOT PRESSURE

2.1 Methods to assess skin blood flow

2.2 Methods to assess plantar foot pressure

2.3 Methods to assess external pressure and skin blood flow

2. CHAPTER 2: A review of methods to assess skin blood flow and plantar foot pressure

2.1 Methods to assess skin blood flow

2.1.1 Introduction

Measuring blood flow, non-invasively, at the level of the microcirculation using laser light and the Doppler effect was developed over twenty-five years ago. This was possible following the development of low-power lasers producing monochromatic light and research into how laser light interacts with moving blood cells by broadening the frequency of backscatter light (Abbot *et al.,* 1993; Stern, 1975). The properties of interest of lasers to study blood flow are its monochromaticity (that is, its narrow range of wavelengths) and its coherence (that is, the light waves contain the same frequency, phase, amplitude and direction) (Shepherd, 1990). However, in the early days little was known regarding the behaviour of light reflection in tissues (Abbot *et al.,* 1993). In a study investigating the measurement of blood flow in animal and human tissue, Bonner *et al.* (1981) produced a linear output from the signal obtained by measuring the laser Doppler shift from reflected light with changes in blood cell flux. The study allowed for the development of the laser Doppler fluxmeter for measuring microcirculatory blood flow. At present, laser Doppler fluxmetry appears to be the most sophisticated method to assess skin blood flow non-invasively and can be used to assess the level of local skin ischaemia (Raamat *et al.,* 2001; Pabst III *et al.,* 1985).

Assessing skin blood flow non-invasively is of great value both for clinical diagnosis and research (Raamat *et al.,* 2001). However, in a clinical setting most tests to assess the risk of ischaemia involve assessing the macrovascular system (for example, checking foot pulses, the ankle brachial pressure index, ultrasound imaging, etc.).

> *"Yet, adequate cellular metabolism is most dependant on capillary haemodynamics and any measurements of the macrocirculation are, at best, only indirect means to estimate ischaemia."* Pabst III *et al.,* (1985).

In a study investigating ischaemic limbs, Pabst III *et al.,* (1985) found that using the laser Doppler fluxmeter, the reactive hyperaemic index and skin perfusion pressure can distinguish between ischaemic and "normal" limbs, even at more proximal levels where the difference in hyperaemic flow and perfusion pressure between the two groups are usually less marked. This suggests that assessing the state of tissue perfusion using the laser Doppler fluxmeter is quite sensitive and is ideal for identifying any tissue ischaemia between test subjects and "normals" (for example, in patients suffering from rheumatoid arthritis and healthy controls).

2.1.2 Laser Doppler flowmetry

There are over ten laser Doppler fluxmeter instruments commercially available and all work on the same principle by estimating skin blood flow optically (Kernick *et al.,* 1999; Bircher *et al.,* 1993; Holloway *et al.,* 1977). The laser Doppler fluxmeter comprises of a laser emitting diode (usually helium-neon). A laser wavelength of monochromatic light produced at 632.8 or 780 nm (depending on the flowmeter used) that is directed onto vascularised tissues by an optical fibre perpendicular to the skin, where erythrocytes account for virtually all-moving structures (Emmanuel *et al.,* 2000; Bukhari, 1993; Bircher *et al.,* 1994; Seifalian *et al.,* 1994; Sundberg, 1984; Engelhart *et al.,* 1983).

> *"Although the vascular bed of, for example, the skin is "visible" from the surface, in the sense that it imparts colour to the skin, light reaches the blood and returns will generally suffer multiple scattering during diffusion through tissues."* Stern, (1975).

Thus, when the laser light collides with the moving particle (that is, a red blood cell within a vessel in the dermal plexus) the speed of the cell determines the frequency of reflected light, whilst the frequency of light

reflected from non-moving structures remains the same (Emmanuel *et al.,* 2000; Bircher *et al.,* 1994, Seifalian *et al.,* 1994; Bukhari, 1993; Sundberg, 1984). Because of the complexities of the peripheral vascular network, with red blood cells possessing different velocities and laser light reaching the blood cells at varying angles, a broad spectrum of varying frequencies is produced (Engelhart *et al.,* 1983). The reflected light is collected by optical fibre(s) and returned to a photo-detector, where the signal is amplified to reach a high signal-to-noise ratio and is processed to determine the frequency shift (Emmanuel *et al.,* 2000; Seifalian *et al.,* 1994; Bukhari, 1993; Sundberg, 1984). Analysis of the spectral components of the backscatter light by an inbuilt microprocessor provides information on cutaneous blood flow (that is, flux) over any desired period of time (Emmanuel *et al.,* 2000; Bonner *et al.,* 1981). It is not possible to calibrate the laser Doppler fluxmeter against an absolute value related to blood flow and hence does not provide measures of blood flow in millilitres per minute, but does provide a relative value of blood flow expressed in arbitrary units of "flux". Flux is linearly related to the speed of moving erythrocytes multiplied by the concentration of those moving red blood cells in the hemispheric volume of tissue measured (Seifalian *et al.,* 1994; Caspary *et al.,* 1988; Sundberg, 1984; Stern, 1975). However Winsor (1987) stated that the values reflect skin blood flow and can be expressed in terms of millilitres of blood flow per 100 grams of tissue per minute. At present, the laser Doppler fluxmeter is not able to distinguish between blood flow from cutaneous arterioles, capillaries, venules and arteriovenules and arteriovenous anastomoses (Bukhari, 1993). In addition Vongsavan *et al.* (1993)[a] carried out an *in vitro* study using animal teeth to investigate if the laser Doppler fluxmeter could distinguish between pulpal blood flow pumped in the same or opposite direction through two cannulae (each having an internal diameter of 0.30 mm and outer diameter 0.63 mm) in the same tooth. Measurements were taken using heparinised blood, pumped using a motorised syringe; first, flowing in the same direction through the blood cannulae and secondly, with blood flowing in opposite directions. The study found that the laser Doppler fluxmeter was insensitive to a difference of 180 degrees in the direction of flow.

2.1.3 Motion artefacts

The signal generated by the single point laser Doppler fluxmeter contains a pulsatile component whose origin may be multifactorial. This may occur due to pulsatile capillary flow during each cardiac cycle, hence there are alternating periods of higher and lower blood flow through the capillaries that are detected by the instrumentation's sensitivity. Alternatively, the pulsatile signal may occur due to the pulsating movement artefact of the tissues, caused by the alternating distension and reduction of the micro-vessels during the heart's diastolic and systolic cycles (Gush *et al.,* 1987). In patients with arterial obstructions proximal to the site been tested, the pulsatile flux was dampened or totally abolished in severe cases (Beinder *et al.* 1992). Motion artefacts may also occur as a result of sensor movement relative to the skin. Cobb *et al.* (2001) overcame the sensor movement artefact problem by restraining the laser Doppler fluxmeter's sensor within a recess cut in the shoe and used double-sided sticky tape to maintain the sensor in skin contact. Furthermore, neoprene rubber straps were used to maintain the foot in contact with the sensor in the shoe. However, care had to be taken when adjusting the straps so as not to over tighten and impose a load on the measuring sensor (Cobb *et al.,* 2001).

The optic fibre cables used by the laser Doppler fluxmeter to transmit the laser beam to the skin and collect the scattered light may produce some non-Doppler effect fluctuations, however these optical fibre line movement artefacts can be effectively reduced. First, by ensuring that the fields of view of the laser emitting fibre and the scatter light collecting fibre do not overlap at the skin surface. This is done by ensuring that the probe tip maintains contact with the skin at all times during data collection and by having separate emitting and collecting fibres. Secondly, by limiting the movement of the speckle pattern emergent from the emitting optical fibre. This may be achieved by selecting a small aperture fibre, preferably of graded-index or monomode type) and/or by dampening the movement of the optical fibre. Aluminium foil crumpled along the length of the optical fibre cable or coaxial copper braiding may be used as a suitable cladding material. Additionally, the cable may be clamped at several positions along its length (Gush *et al.,*

1987). Other authors have obtained similar dampening stabilisation of the cables by injecting Vaseline® into a plastic catheter containing the fibre optic cables. Other precautions should also be taken into account when designing a laser Doppler fluxmeter instrument to reduce motion artefact include, optimising the angle at which the laser beam is emitted into the optic fibre and into the tissues at the distal fibre end, the method used to arrange the optical fibres in bundles and the quality of the optical fibre end surface (Oberg, 1990). Some systems (for example, Perimed PF2 instrument, Perimed, Sweden) electronically detect and reject movement artefacts from optical fibres. Other methods of reducing motion artefact from optical fibres are to design an integrated probe with the emitting laser diode and receiving photodiodes within the probe (Oberg, 1990).

In dynamic studies of recording skin blood flow in the plantar aspect of the foot during walking, a movement artefact occurring as a result of compression of tissues during standing and occasionally during turning at the end of the test pathway, was noted when both the magnitude and period of the load signals differed from that of "normal" walking (Cobb *et al.,* 2001). This may have occurred as a result of shear forces during turning but were not measured.

2.1.4 Biological zero

There are some differences between the mathematical modelling and instrument design of the laser Doppler fluxmeter and current understanding of the physiology of cutaneous blood, since a residual signal is generated by vasomotion activity within the tissues when the arterial flow is completely occluded. This flow independent signal is called the "biological zero" (Fagrell, 1994; Abbot *et al.* 1993). In animal models (for example, the hamster skin) it has been demonstrated that during complete circulatory arrest a laser Doppler fluxmeter signal was achieved from the skins' vascular bed (Fagrell, 1994). Some authors recommend that, if possible, this signal should be subtracted from all observations in clinical practice (Fagrell, 1994; Abbot *et al.* 1993; Caspary *et al.* 1988). A study into the origin of biological zero in various different types of unperfused animal

tissues found the signal to be the lowest in adipose tissue followed by skin; the bowel recorded the highest laser Doppler fluxmeter signal which was 3.5 times greater than that recorded at the skin (Kernick *et al.*, 1999). Differences in biological zero between an integrated probe (containing the laser emitting diode and photo-sensor within the probe) and the standard probe (using optic fibres to transmit and receive the scattered light between the probe and unit) found the latter to register less for the proportion of biological zero value of the laser Doppler output (integrated probe 31% against standard probe 52% - $p<0.01$) (Norman *et al.*, 1995). This difference may possibly be accounted for by construction differences between the probes.

Equation for percentage of biological zero	***Occluded SBF***	***Resting SBF***	***% BZ output***
$\% \text{ Output of BZ} = \frac{\text{SBF following arterial occlusion} \times 100}{\text{Resting SBF}}$	25 AU	25 AU	100 %
	25 AU	31.25 AU	80 %
	25 AU	50 AU	50 %
	25 AU	100 AU	25 %
	50 AU	25 AU	200 %
	50 AU	31.25 AU	160 %
	50 AU	50 AU	100 %
	50 AU	100 AU	50 %

Table 2-1: The effects of increasing or decreasing the skin blood flow post-arterial occlusion and the resting skin blood flow. The formula for the percentage output of biological zero is shown on the left and examples of various resting skin blood flow and biological zero values are shown on the right (SBF - skin blood flow; BZ - biological zero) (Caspary *et al.*, 1988). AU refers to arbitrary units.

Caspary *et al.* (1988) stated that the flux-independent biological zero could amount up to 80% of the total signal output in patients with severe peripheral arterial occlusive disease studied. This value may be accounted in that the subjects with severe peripheral arterial occlusive disease may have had a lower resting blood flux. If the biological zero remains about the same in all test subjects, then when calculating the percentage the lower the skin blood flow flux the higher the biological zero percentage output (see Table 2-1). Kernick *et al.* (1999) carried out an *in vitro* model and showed that when vessels were empty of blood the biological zero signal was higher than when the vessels where full of static blood. The study suggested that Brownian motion of erythrocytes and their aggregates was negligible on the

biological zero signal and that in fact, they masked the significant biological zero signal from the deeper interstitial tissues. Such findings may also explain the high biological zero percentage output that Caspary *et al.* (1988) found in patients with severe peripheral arterial occlusive disease if deeper interstitial tissues influenced the post occlusive measurement of biological zero as a result of collapsing perfused vessels upon the application of the pressure cuff. A combination of both, an increased biological zero value and low resting skin blood flow value, would have resulted in such high percentage biological zero output (see Table 2-1). In the oedematous ischaemic feet of patients suffering from diabetes mellitus the biological zero signal may be related to the inflammatory process of the skin (Fagrell, 1994).

In a study of five male cadavers (time of death 8 to 24 hours prior to data collection) Caspary *et al.* (1988) found a laser Doppler fluxmeter reading above instrument zero in the various sites tested. Upon local heating of the cadavers' skin, using a heating element within the probe holder and raising skin temperature to approximately 37°C, the output value increased further. Upon cooling the signal returned to its baseline value. Caspary *et al.* (1988) suggested that the origin of the increasing biological zero signal may be related to Brownian molecular motion within the tissues at the sites being tested. Furthermore, when the unperfused cadavers' skin at 36.5-37°C was compared with *in vivo* skin post-arterial occlusion with a cuff (for 10 healthy volunteers and 15 test subjects with severe peripheral arterial occlusive disease), the values were all within the same range (Caspary *et al.* 1988). In patients with severe peripheral arterial occlusive disease and in young or elderly healthy controls, Abbot *et al.* (1993) found the biological zero values to be similar. Kernick *et al.* (1999) also found a relationship between red blood cells and temperature resulting from Brownian motion in an *in vitro* experiment. Cobb *et al.* (2001) developed a laser Doppler fluxmeter capable of measuring dynamic cutaneous blood flow and found that the pre-dynamic testing, following cessation of blood flow to the foot using a cuff at 200 mmHg to healthy test subjects, resulted in a typical fall in the signal output value of 10 mV. This output signal was attributed to Brownian motion within

the tissues and instrumentation noise (Cobb *et al.,* 2001). The application of the cuff to arrest circulation may cause displacement of arterial blood and venous congestion, especially if the cuff has been inflated slowly, affecting the concentration of interstitial scattering cells. Although approximately two minutes post arterial occlusion in *in vivo* studies it is unlikely to be significant (Kernick *et al.,* 1999).

In a study investigating the biological zero in neonates and adults, Norman *et al.* (1995) found no significant differences between obtaining the biological zero through cuff inflation or direct application of pressure to the probe attached to the skin on the heel ($p<0.001$).

The origin of biological zero may stem from a number sources:

A source may arise shortly after arterial arrest to the site tested and is a consequence of shunting of blood between the various compartments of the skin's microcirculation in response to differences in local pressure or residual vasomotion as a result of a reduction in the diameter of the larger vessels (Kernick *et al.,* 1999; Abbot *et al.* 1993).

Another source that probably produces a more marked biological zero signal occurs where more interstitial fluid is present (for example, at the sites of inflammation) and is due to extra-vascular activity resulting from fluid and cellular movement within the interstitial space; there is interstitial fluid movements due to convection and currents as it drains into the lymphatic system or is re-absorbed into the capillaries, Brownian motion of cellular debris/interstitial macromolecules and active migration of lymphocytes and/or monocytes within the tissues (Kernick *et al.,* 1999; Abbot *et al.* 1993).

Failure of arterial circulatory arrest caused by collateral circulation through bone (Kernick *et al.,* 1999).

Active motion within the vascular vessels unrelated to blood flow. Such movement may occur as a result of erythrocyte aggregation or sedimentation or Brownian motion within the vessel. However, this would not occur until approximately 15 minutes of vascular stasis

and thus is unlikely to influence post-occlusive in *in vivo* tissue experiments but may influence *in vitro* unperfused tissue (Kernick *et al.*, 1999).

2.1.5 Measurement depth

The depth of sensitivity of the laser beam depends on the geometry of the tissue bed, emitting and receiving fibre optic separation within the probe and the wavelength of the laser light (Abu-Own *et al.*, 1995; Gush *et al.*, 1984; Obeid *et al.*, 1988). Near-infra-red light penetrates Caucasian skin to approximately 1200 μm. Red light of 600 nm reaches a depth of approximately 550 μm and green light approximately 150 μm (Anderson *et al.*, 1981). In a study investigating depth discrimination of different laser Doppler fluxmeter lasers Obeid *et al.* (1988) found that green laser lights show a significantly reduced response to blood flow. This may be the case because green laser light has a lower frequency and penetrates tissue less. Since rate of skin blood flow is related to diameter of arterial vessel lumen and the more superficial the vessel the smaller its diameter and lesser the flow, this may explain the reduced response to blood flow. Gush *et al.* (1984) studied the variation of probe intensity with optic fibre probe separation. The skin was illuminated in turn with green (argon ion laser used at 514.5nm), blue (argon ion laser used at 488.0 and 476.5 nm) and red (helium-neon laser used at 632.8 nm) at increasing optic fibre separation within the probe. The study concluded that penetration depth is dependant on laser wavelength and optic fibre separation (of emitting and receiving fibres) within the probe. For normal probes with separation of less than 2 mm the authors stated that the papillary loops and the most superficial venous plexus of the dermis seems to be sampled. The study did not recommend the selection of laser light at shorter wavelengths for superficial vessel selectivity.

The depth to which the laser beam penetrates varies and is related to the type of tissue being studied, the laser wavelength and the probe type used (Seifalian *et al.*, 1994; Bonner *et al.*, 1990; Gush *et al.*, 1984). In a study to establish reference values for skin, hourly and daily variations and the

effects of sublingual nitroglycerin on skin blood flow, Sundberg (1984) using the Periflux laser Doppler fluxmeter (Periflux PF 1D, Perimed, Sweden) reported a measuring surface of 1.2 mm^2 and a penetration depth of approximately 1 mm. In a different study Holloway *et al.* (1977) suggested the penetration depth of the laser Doppler fluxmeter to be approximately 1 to 1.5 mm. In a theoretical study investigating the model for laser Doppler flowmetry to measure blood flow in tissue, Bonner *et al.* (1981) reported the instrument to have a temporal resolution of approximately 100 msec and a spatial resolution of 1 mm.

A number of studies have investigated aspects relating to optic fibres and tissue penetration depth. Stern (1990) reported that a two-fibre probe system detects flow at a greater distance than systems using one optic fibre only. Gush *et al.* (1984) investigated the effects of optical fibre separation within the probe for the two-fibre system and reported that distance between the emitting and receiving optic fibres is related to measuring depth, with the greater the distance between the fibres, the greater the penetration depth. In addition, the laser power will also affect the penetration depth (Bonner *et al.*, 1990).

In human teeth the depth of penetration of laser Doppler fluxmeters (Periflux PF3, Perimed, Sweden; and MBF3D/42, Moor Instruments, UK) was 3.1 mm (including enamel and dentine) for an *in vitro* study were the flow of diluted human blood through the pulp cavity was controlled. Differences between skin and the more transparent nature of dental tissues may explain the depth of penetration in teeth to be greater than that in skin (Vongsavan *et al.*, 1993[a]).

Laser Doppler fluxmetry seems to measure nutritional blood flow in the papillary capillary loops as well as thermoregulatory flow in the subpapillary arterio-venous shunts (part of the skin's thermoregulatory control) (Abu-Own *et al.*, 1995). Underlying muscle tissue does not influence the cutaneous blood flow using the laser Doppler fluxmeter (Saumet *et al.* 1988). Under conditions of pressure loading, any possible small contribution made by deeper vessels in the subpapillary with higher blood flow rates,

may be increased as the tissues are compressed and the proximity of the laser Doppler fluxmeter sensor to these vessels increases. However, in the forefoot large vessels are anatomically located in recesses between the metatarsal heads and thus, the head of the metatarsals acts as the upper boundary for the sampled volume of tissue containing only the small cutaneous vessels of interest (that is, arterioles and capillaries of size below 100 μm) (Cobb *et al.,* 2001). Similarly, on the plantar aspect of the calcaneus, the calcaneal arteries and lateral and medial plantar arteries pass distal to the medial and lateral tubercles of the calcaneus (Romanes, 1999). Thus, the centre of the plantar aspect of the calcaneus will not be affected by blood flow in large vessels when studying the effects of plantar foot pressure on skin blood flow.

2.1.6 Validation studies

Holloway *et al.* (1977) found a close correlation ($r=0.89$; $p<0.001$) between the laser Doppler fluxmeter and the ^{133}xenon clearance blood flow in the forearm of "normal" subjects. However, one should bear in mind that each of the above techniques measures different parameters. With ^{133}xenon clearance the speed of blood flow is dependant on the rate of absorption of a radioisotope by the microcirculation and is affected by the injection depth and diffusion of the tracers through the tissues between the capillaries and injection site. The ^{133}xenon radioisotope is also fat-soluble making the method more difficult to analyse. The laser Doppler fluxmetry technique does not measure blood flow *per se* but moving red blood cells (Holloway *et al.,* 1977). In addition, the laser Doppler fluxmeter measures blood flow in capillaries and arteriovenous anastomoses whilst the ^{133}Xeron washout technique measure blood flow in the capillary network only (Engelhart *et al.,* 1983). Thus, both methods will always show a slight difference during correlational studies because they are measuring different but related parameters.

Two types of laser Doppler fluxmeter instruments were compared by Netten *et al.* (1993). The Periflux PF1d (Perimed, Sweden) uses a helium-neon laser (wavelength laser output 632.8 nm) with optic fibre wires emitting and

receiving the scatter light between the probe and the unit. In contrast, the Diodopp instrument (Applied Laser Technology, Maarheeze, Netherlands) uses a diode laser (wavelength laser output 780 nm) and no optic fibres with the laser source and detectors integrated in the probe. Both systems proved equally valuable for measuring skin blood flow, although the Diodopp laser Doppler fluxmeter seemed to produce slightly better reproducible results. However, the Diodopp's post-occlusion hyperaemic response was attenuated. Norman *et al.* (1995), using the laser Doppler fluxmeter (Periflux PF2B, Perimed AB, Perimed, Sweden), also found more reproducible results of biological zero in neonates with an integrated probe than a standard probe. In a different but *in vitro* study investigating pulpal blood flow of human and animal teeth Vongsavan *et al.* (1993[a]) reported, under the same experimental conditions, differences between the two laser Doppler fluxmeters tested (that is, the MBF3D/42, Moor Instruments, UK; and the Periflux PF3, Perimed, Sweden). The difference between the two fluxmeters tested was in the range of 1.8 to 2.9 times greater, with the Moor instrument recording higher values than the Periflux.

A number of authors have used the laser Doppler fluxmeter to measure digital, ankle or brachial artery and skin perfusion pressure and compared it with other more conventional systems (Fisher *et al.,* 1995; Beinder *et al.,* 1992; Andersson *et al.,* 1986). A study was carried out to compare the laser Doppler fluxmeter for measuring distal blood pressure with the conventional strain plethysmography gauge technique. The strain gauge plethysmography has been used for over 40 years and works on the principle that arterial blood inflow, measured by a mercury strain gauge during slow release of arterial occlusion by a pneumatic cuff, will cause an increase in the volume of the segments in arms or legs. During simultaneous measurement of the distal systolic blood pressure of the hallux and ankle, using both techniques, high correlation coefficients were achieved ($r=0.98$ and $r=0.99$ respectively). However, in the results section the p-value is stated as $p>0.05$ (Andersson *et al.,* 1986). The p-value is described as the probability that the observed results, or a more extreme outcome, would have occurred by chance when the null hypothesis is true.

When p is greater that 0.05 the null hypothesis cannot be rejected (Petrie *et al.,* 2000; Altman *et al.,* 1983; Altman, 1980). Thus, the study by Andersson *et al.* (1986) may be interpreted in two ways: first, that the null hypothesis is true and there is no correlation between the two methods tested; and secondly, that the authors made a simple typing mistake and the p-values are actually below 0.05, thus, a correlation between the two methods used exists. In which case, when compared to the strain plethysmography gauge technique, the laser Doppler fluxmeter seems to be more sensitive in measuring segmental distal systolic blood pressure in the low-pressure range.

The laser Doppler fluxmeter technique for measuring segmental systolic blood pressure was compared with the Doppler ultrasound method. Good correlation coefficients of 0.96 were shown between the two methods (Beinder *et al.,* 1992). However, one should note that although both systems use the Doppler effect to measure circulation, one using sound waves and the other monochromatic laser light, the ultrasound Doppler method measures blood flow in deep lying vessels whilst the laser Doppler technique measures blood flow in superficial vessels (Bukhari, 1993). When measuring systolic blood pressure both methods measure the start of blood flow following circulatory arrest distal to the cuff, in vessels at different depths, with temporal variations in the commencement of blood flow in vessels distal to the cuff almost the same. This may suggest why the two systems measuring blood flow at different spatial parameters were correlated.

Almond *et al.* (1988) investigated the relationship between the laser Doppler fluxmeter and the photoplethysmograph signal for measuring skin blood flow in the pulp of the finger. Photoplethysmography is widely used to measure changes in peripheral circulatory change with an alternating current signal (the ac photoplethysmograph used as an indicator of changes in blood flow) and the slowly changing current (the dc photoplethysmograph used to measure changes in blood volume or flux) (Kamal *et al.,* 1989; Almond *et al.,* 1988). However, the dc photoplethysmograph has been

correlated with the venous occlusion strain gauge plethysmography suggesting that the dc component of the photoplethysmograph signal also reflects changes in blood flow (De Trafford *et al.*, 1984). Although, the dc photoplethysmograph may only reflect changes in blood flow when venous filling is low and the return remains unrestricted (Almond *et al.*, 1988; De Trafford *et al.*, 1984). The laser Doppler fluxmeter and the dc photoplethysmograph correlated well when measuring skin blood flow on the pulp of the finger under conditions of low venous filling with unrestricted venous return. However, the laser Doppler fluxmeter method provided a reliable measure of skin blood flow irrespective of the degree of venous filling (Almond *et al.*, 1988).

Seifalian *et al.* (1994) compared laser Doppler perfusion imaging (Lisca Development, Moor Instruments Limited, UK), that works on the same principle as the laser Doppler fluxmeter but there is no skin contact with the laser Doppler imager aimed at the skin from a distance of approximately 30 to 100 centimetres depending on the area being scanned. The system scans a bigger area, than the laser Doppler fluxmeter. The results showed a close correlation between the laser Doppler imager and the laser Doppler fluxmeter ($r=0.960$; $p<0.01$).

In a study investigating the reliability of the laser Doppler fluxmeter to measure the microcirculation of the pulp of the hallux, the vaginal and rectal mucosa and the effects of the menstrual cycle on these, Emmanuel *et al.* (2000) found the laser Doppler fluxmeter to be a reliable method to assess these.

The laser Doppler fluxmeter was compared with the hydrogen clearance technique to measure blood flow of the intestinal mucosa in animals. The hydrogen clearance technique measures blood flow by the rate of disappearance of hydrogen from perfused tissues, which is determined by the rate of blood flow. A linear correlation was achieved for both techniques ($r=0.73$; $p<0.0001$) (Kvietys *et al.*, 1986).

2.1.7 Conclusion

The laser Doppler fluxmeter is a valid system to measure skin blood flow and has been successfully correlated with other more established methods of measuring skin blood flow. It is also extensively used both in research and clinical practice to study skin blood flow. Although some limitations seem to exist, these can be minimised with the design of the instrument and by controlling experimental variables. With regards to penetration depth, the laser's ability to penetrate soft tissue seems to vary depending on the optical qualities of the tissues investigated, but in skin the penetration depth of the most widely available instruments seems to be approximately 1-1.5 mm. Laser Doppler fluxmetry is a valid method that could be integrated with a pressure system to evaluate the effects of external pressure on skin blood flow.

2.2 Methods to assess plantar foot pressure

2.2.1 Introduction

The human foot spends most of its time in contact with the external environment with its function controlled by muscles, both intrinsic and extrinsic. During human walking an upright position is maintained with plantar foot pressure transmitted through the skin onto deeper structures. These plantar foot pressures occur as a result of ground reaction force. Ground reaction force is described as an equal and opposite force to the body's impact on the ground that occurs as the foot makes ground contact (Abboud *et al.,* 1996; Mueller, 1995). Thus understanding the functionality of the foot to ground interface is vital.

At present, there is a wide range of measuring equipment available to determine the magnitude and direction of static and dynamic forces. Some methods only give visual impressions whilst also others measure vertical forces, pressure and more recently a few measure shear forces. However, variations between studies about the unit of measure and direction of the force create varied outcomes depending on the type of sensors used and hence makes comparing studies difficult.

2.2.2 Foot-to-ground plantar foot pressure systems

Previous reviews detail the development of early foot-to-ground systems (Abboud *et al.,* 1996; Lord, 1981). Modern systems use force plates and allow for plantar foot pressure to be recorded electronically. Lord (1981) describes "Force Plates" as flat plates capable of measuring the components of force applied to their top surface. Force plates are usually set flush within a walkway and are capable of capturing the force distribution during one single step of the gait cycle. Because force plates are made up of a number of smaller sensors distributed within the plate and each sensor can register an individual recording of force at that site, a force map can be obtained (Lord, 1981). Over the years a number of force plate systems have been developed. The first force plate systems used mechanical sensors to register applied force (for example, the *Basler's harp-like instrument* and more recently the Plastic Pedobarograph, both an optical and mechanical force plate, since it is composted of an array of 10 mm square-section vertical clear plastic rods resting on a rubber sheet) (Lord, 1981; Basler, 1927). However, more modern systems use light, piezo-metric transducer systems, strain gauges, etc. to convert applied load to an electrical impulse that is then processed by a computer into force and pressure. The early systems were composed of few sensors and suffered from poor resolution but with advances in technology better higher-resolution pressure platforms were developed.

The modern dynamic Pedobarograph follows the principle described by Elftman in 1934 using a light source and a series of dots and squares on a glass plate that was recorded using a cine-camera (Abboud *et al.,* 1996; Franks *et al.,* 1983[b]; Duckworth *et al.,* 1982; Lord, 1981). The modern dynamic Pedobarograph consists of a thin sheet of opaque plastic, that makes contact with the subject's foot, with a glass plate beneath it, that is illuminated at its edges with strips of light (Urry, 1999; Abboud *et al.,* 1996; Bradley *et al.,* 1986; Franks *et al.,* 1985; Duckworth *et al.,* 1982; Lord, 1981). When force is applied (for example, by walking or standing) intimate contact occurs between the opaque plastic sheet and the illuminated glass plate, which results in an alteration of the total internal reflections within the

glass. When viewed from below the pattern of light intensity is proportional to the applied distribution of pressure under the area of foot contact (Urry, 1999; Bradley *et al.,* 1986; Franks *et al.,* 1985; Duckworth *et al.,* 1982). A mirror angled at 45 degrees below collects the refracted light from the sandwiched glass under load and allows a video camera, connected to a computer, to record the data for analysis with plantar foot pressure being proportional to the amount of light scattered (Urry, 1999; Abboud *et al.,* 1996; Bradley *et al.,* 1986; Franks *et al.,* 1985; Franks *et al.,* 1983[b]; Duckworth *et al.,* 1982; Lord, 1981). The maximum sampling rate of the system is 30 Hertz (Abboud *et al.,* 1996; Franks *et al.,* 1983[a]; Franks *et al.,* 1983[b]; Duckworth *et al.,* 1982). The modern dynamic Pedobarograph has the disadvantage that it is not portable (Urry, 1999; Duckworth *et al.,* 1982). The pedobarograph can be calibrated by applying known loads on the recording platform.

The Musgrave plantar foot pressure platform system (Musgrave Park Hospital, Belfast, UK) used a matrix of 32 by 16 conductive foam rubber transducers making calibration difficult, thus the foam was replaced with Force Sensing Resistor material providing a matrix of 2,048 sensors (each sensor 5 by 5 mm^2) (Abboud *et al.,* 1996). The new system can be calibrated statically and dynamically using a hydraulic piston and the system has a sampling speed of 113,777 sensors per second and a sensitivity of 0.1-17 Kg/cm^2 (Abboud *et al.,* 1996). However, Young *et al.* (1993) found that the Musgrave saturates above 15 kg/cm^2 giving incorrect results above this value. The Musgrave sensors also suffer from some level of creep with sensors taking some permanent defrmation.

Another platform system is the EMED system (Novel GmhH, Germany). There are various EMED platforms available, which are produced for a variety of applications and they differ in weight, size, number of sensors, sampling frequency and resolution (see Table 2-2) (Abboud *et al.,* 1996; www.novel.de, 2004). The EMED system uses capacitive sensors that measure the electrical parameter of the effects of mechanical deformation under load of two plates with the closer together they become as the

resultant changes in capacitance (Lord, 1981). Similarly to the Musgrave the EMED dielectric sensors also suffer from some level of creep.

Platform type	Sensor area	Sampling frequency	Sensor resolution
EMED-SF-1	500 × 300 mm²	Max 100 Hz	1 sensor per cm²
EMED-SF-2	645 × 341 mm²	Max 70 Hz	2 sensor per cm²
EMED-SF-4	570 × 322 mm²	Max 50 Hz	4 sensor per cm²
EMED-SF-Tires	420 × 417 mm²	Max 40 Hz	4 sensor per cm²
EMED-SL	360 × 190 mm²	Max 50 Hz	2 sensor per cm²
MINI-EMED	350 × 160 mm²	Max 16 Hz	3 sensor per cm²
MICRO-EMED	85, 170, or 256 sensors	100, 50, 20 Hz respectively	9 sensor per cm²

Table 2-2: The types of EMED platform systems available (Abboud *et al.*, 1996).

Piezo-electrical crystals offer the potential for accuracy, good dynamic response and sensitivity. Piezo-electrical crystals, when deformed, generate an electrical charge along certain crystallographic axes and can be measured with a suitable amplifier. The electrical impulse may then be equated to the proportion of force applied. (Lord, 1981). However, the piezo-electrical crystals are as sensitive to temperature as applied pressure. The *Kistler Force Plate* (Kistler Instruments Ltd., Switzerland) transducer consists of four load cells, one at each corner, and is capable of measuring applied forces and moments along three orthogonal directions (Abboud *et al.,* 1996; Lord, 1981).

Other types of platform systems using strain gauge sensors have also been reported (Lord, 1981). A strain gauge consists of one or more foils glued to a short beam that bends slightly upon the application of force. Thus, when the force acts on the beam and the beam bends the foil increases in length and its thickness decreases. The changes in the shape of the foil cause a change in the resistance of the material. By calibrating the change in resistance against applied loads the strain gauge sensor may be used to measure the magnitude of applied forces (Winn, 1994). Strain gauges have also been used for in-shoe systems (Urry, 1999; Abboud *et al.,* 1996; Alexander *et al.,* 1990; Lord, 1981).

Finally, the Podinamic platform systems (Zeno Burratto SpA, Italy) consists of a Force Sensing Resistor material sandwiched between two layers of polymer colaminates with interlaced electrodes. There are three different platforms (the PS1, PS2 and PS3) with the size of individual sensors being 0.8×0.8 mm^2 and a sensitivity range of 250 g/cm^2 (25 kPa) to 10 kg/cm^2 (1000 kPa) with a sampling rate of 250 Hertz. The PS2 and PS3 are also capable of producing multi-step analysis up to a maximum of three (Abboud *et al.,* 1996).

Platform systems do not mark areas of pressure anatomically, for example, EMED Systems (whose large sensors creates difficulties when marking specific areas of high pressure) (Resch *et al.,* 1995; Hughes *et al.,* 1993). As a result, accurate referencing of sites of plantar pressure to specific anatomical sites is difficult and can only be done visually. Thus, making the subject walk over the pressure platform first, followed by a second recording of skin blood flow would be floored with positional errors as pinpointing the exact anatomical pressure site of two different recordings, one for skin blood flow and one for plantar foot pressure, is not accurate enough for studies into the effects of plantar foot pressure on skin blood flow. Also, because skin blood flow and plantar foot pressure would not be recorded in real time the plantar foot pressure or skin blood flow recorded in two different sessions may be different. Thus, if using a platform system, the skin blood flow sensor would have to be embedded into the platform for simultaneous recordings of skin blood flow and plantar foot pressure. However, all these types of platform systems failed to provide any information regarding in-shoe pressures, which would provide more meaningful data representative of shod populations (McPoil *et al.,* 1995).

2.2.3 In-shoe systems

Over the past decadal great interest has augmented amongst researchers and clinicians over methods of measuring in-shoe pressures, that is, measuring the forces and pressures that occur between the interface of the plantar surface of the foot and the insole of the footwear. Thus, the distribution of multi-step plantar foot pressure during normal activity for shoe

wearing subjects may be recorded. The first in-shoe system to measure plantar foot pressure was developed by Etienne Jule Marey and Gaston Carlet (1830-1904). The device consisted of pressure sensitive shoes connected to a recording device. The subject wore special shoes with an air chamber in the mid-metatarsal area of the foot and data collection occurred with no interference by the investigator. Weight on the shoe caused the in-shoe air chamber to increase in pressure and was recorded by a device carried on the back. Carlet improved the device by adding a heel and forefoot chamber; however the devices did not quantify pressure (Johanson, 1994; Alexander *et al.,* 1990). Later the popular Harris and Brand Mat was initially adapted for this purpose by cutting the mat into an insole and placing the inked mat in the shoe with a paper cover on top. Advancements in technology have allowed for computerised systems that measure in-shoe dynamic plantar pressures like the FScan and Pedar systems. Literature reports that computerised in-shoe dynamic plantar pressure measuring systems, using present technology, may record scientifically valid and reliable results (Resch *et al.,* 1995; Cavanagh *et al.,* 1992).

As technology progressed, from as early as 1947, some experimental systems have been developed to measure in-shoe plantar foot pressure using electrical/computerised methods (for example, using capacitive pressure pads, piezoelectric discs or strain gauges either taped to anatomical sites on the feet or incorporated inside the shoe to measure dynamic in-shoe plantar foot pressure) (Abboud *et al.,* 1996; Alexander *et al.,* 1990; Bradley *et al.,* 1986; Lord, 1981). As pressure sensors became smaller and computers faster with higher sampling rates, modern dynamic in-shoe plantar foot pressure-measuring systems became commercially available.

The dynamic in-shoe FScan system (Tekscan Inc., Boston, Massachusetts) uses an insole with a matrix of Force Sensing Resistor material pressure sensors. The micro-thin insole (0.2 mm) is composed of a matrix of 960 capacitive pressure sensors at an interval of 5 mm (Urry, 1999; Abboud *et al.,* 1996; Mueller, 1995). Each insole can be trimmed to fit the shoe size

with a sensing range per sensor of 56 to 868 kPa and a sampling rate of 100 Hertz (Abboud *et al.,* 1996). Unfortunately, the thin nature of the insoles makes them prone to wrinkling as they are inserted into the shoe or when walking. In addition, the system suffers from calibration problems and the sensitivity of the insoles declines with use by as much as 20.5% when used 12 times (Urry, 1999; Abboud *et al.,* 1996). In a study, Birke *et al.*, (1994) found that repeated measures to decrease by approximately 7% after 7 trials. The FScan System was used by Rose *et al.* (1992) and found that at around 30 gait cycles, in-shoe sensor wear gave poor results questioning the system's reliability. McPoil *et al.,* (1995) found that the FScan insole, although it shows a linear correlation with applied load, the system has a large amount of error, especially at pressures exceeding 200 kPa. In addition, when masking an area the FScan software (version 3.622) uses the total area being masked, irrespective of the number of sensors active, to work out the pressure distribution (McPoil *et al.,* 1995). Thus, the calculated pressures may be lower than other systems since the software includes the area of inactive sensors when calculating the pressure distribution within the masked site.

The in-shoe Pedar Plantar Pressure Measurement System uses insoles two millimetres in thicknesses that are quite flexible. The insoles contain up to 256 capacitive sensors. The sensors work by measuring the change in capacity that occurs when the distance between the two conducting wires, which is separated by an insulating layer or dielectric, changes as a result of applied load. By calibrating the changes in electrical output to various increasing loads (that is, vertical forces) a look up table or chart is produced. The pressure acting on each sensor is then calculated by the equation: pressure acting on sensor = applied force / area of sensor (Finch, 1999; Urry, 1999; Alexander *et al.,* 1990). The system samples at a rate of 50 Hz thus, allowing for accurate data to be collected when walking (Pedar Standard Manual, 1997; Cavanagh *et al.,* 1992). The in-shoe Pedar System also provides easy and accurate calibration with each sensor in the matrix calibrated independently prior to commencing recording (Pedar Standard Manual, 1997; Kernozek *et al.,* 1996). McPoil *et al.,* (1995) found

the insole capacitive sensors to produce a linear response to applied loads with a minimum margin of error, especially at high pressures. The sensor insole also demonstrated less creep than the FScan insole upon the application of continuous and constant pressure (McPoil *et al.,* 1995). However, when using the Pedar Plantar Pressure Measurement System the accuracy of results during dynamic studies may be affected because capacitive sensors suffer from an electrical time delay (Lord, 1981).

2.2.4 Advantages and disadvantages of pressure systems

In-shoe plantar pressure measurement systems are capable of measuring pressures at the interface between the shoe (or orthotic) and the plantar aspect of the foot during a given functional activity. It also provides the clinician or researcher with a better understanding of the effects of speciality footwear and foot orthotics on foot biomechanics (McPoil *et al.*, 1995; Mueller, 1995). Thus, by measuring within the shoe, the clinician or researcher can perform an objective examination of the therapeutic effects of the footwear, or hosiery modifications, or foot orthoses design, or screening for potential foot pathologies (Kernozek *et al.*, 1996). However, one should bear in mind that when shoes are worn they might have an influence on recorded pressure values. In a study, Corrigan *et al.* (1993) found that varying heel height increases forefoot pressure and moves centre of pressure medially. Rose *et al.* (1992) found that soft, flexible shoes gave lower readings than stiffer, firm shoes. The soft sole cushions, deforming and recovering quickly on application and removal of the force, results in reduced plantar pressures (Blakeman, 1985).

In-shoe systems have overcome some of the disadvantages associated with platform measuring devices. For example, the Musgrave System that measures plantar foot pressures between the foot and ground (Bennett *et al.*, 1993). When using in-shoe techniques instead of pressure measuring platforms, the need for "targeting" is eliminated, and multiple step analysis can be measured bilaterally (McPoil *et al.,* 1995; Cavanagh *et al.*, 1992). "Targeting" occurs when the subject being tested alters their walking pattern so they can make contact with the force plate (McPoil *et al.,* 1995). Thus in-

shoe systems are capable of measuring consecutive footsteps with the foot in its accustomed surroundings, allowing the researcher to evaluate gait rather than isolated footsteps (Akhlaghi *et al.*, 1994). As a result, more robust statistical analysis of relevant parameters may be obtained. The in-shoe measurement system also allows for a more versatile study of given activities other than just level locomotion (Cavanagh *et al.*, 1992).

When comparing the various systems it is important to note the sampling rates, as these will have a bearing on the speed at which data is collected, with the faster pressure sensor-sampling rate giving more accurate results, e.g. MICRO-EMED 20 Hertz and the dynamic Pedobarograph up to a maximum of 30 Hertz. In this example, 10-Hertz difference between the systems means that studies using the dynamic Pedobarograph would yield more accurate results (Abboud *et al.,* 1996; Hughes *et al.,* 1993).

Generally in-shoe pressure measurement systems conform to several important equipment characteristics. The sensors or transducers, used in the construction of the insole, must be small, thin and flexible enough to fit in the shoe without affecting the actual measurement (Urry, 1999; Kernozek *et al.*, 1996; Mueller, 1995; Cavanagh *et al.*, 1992). However, the hot, humid, and often contoured environment can affect the sensors' performance. In addition, another disadvantage is that the sensor insoles are more prone to damage because as the sensor cables exit the shoe they can become bent or stretch. Finally, secondary damage may occur as a result of repeated or excessive loading in the same regions (for example, the heel and the forefoot) (Finch, 1999; McPoil *et al.,* 1995). To overcome the effects of wear on the sensors giving erroneous values the pressure sensors should be validated regularly by bench testing the sensors, using the calibration equipment, against known standards and were necessary recalibrating the sensors.

Force plates are placed and remain on the ground, irrespective of foot movement, throughout the data recording process. This means that vertical forces can be measured more accurately. However, with in-shoe systems measuring vertical forces are more difficult. This is because when the

flexible sensor insole is placed in a shoe the position of the shoe in relation to space can significantly affect the direction of forces acting on the pressure sensor. Thus, with in-shoe systems the position of the sensor insole within the shoe relative to the ground prevents the measurement of "true" vertical force (translated to pressure). This may be more apparent during heel strike, heel lift and toe off during the stance phase of gait. Finally, when the sensor is placed over an orthotic device, the curvature of the device could affect the direction of the measured force vector (Finch, 1999; McPoil *et al.,* 1995). An in-shoe device that can not only measure vertical force but also shear would solve these issues.

A platform or in-shoe system with a high sampling rate is desirable. A high sampling frequency and resolution is required in order to capture data during dynamic activities. Walking activities require at least 50 Hertz, with sporting activities needing 100-200 Hertz. In order to visually associate areas of pressure with anatomical sites the resolution must be quite high (Kernozek *et al.*, 1996; Mueller, 1995; Cavanagh *et al.*, 1992).

Some in-shoe systems use a trailing cable and this may alter the test subject's gait. This has been overcome by some systems (for example, the Pedar plantar foot pressure system) by using a PCMCIA memory card data logger or a telemetry based version (Finch, 1999). However, regardless whether the in-shoe system has a trailing cable or not, the test subject is required to wear a data collection pack and this has the potential to affect the subjects normal gait (Finch, 1999). To minimise the effect of the data collection pack on gait, Santos *et al.,* (2001) placed the pack in the midline of the lumbar region on the test subjects' to avoid interference with the transmission of mass during walking.

Both foot-to-ground and in-shoe systems are liable to postural sway problems when measuring static plantar foot pressure. Up to 30% measurement variation has been reported in foot-to-ground pressure patterns in a "normal" group of children (Bradley *et al.,* 1986). Bradley *et al.* (1986) found postural sway to be most significant along the sagittal plane.

Spatial distortion occurs in devices using elastic mats (for example, devices used in kinetography or pedobarography) as the mats apply a smoothing filter and reduce the fine detail in the pattern of pressure distribution (that is, mats with greater elastic properties or less dense materials will distort more that mats with less elastic properties or more dense materials; in addition, thicker mats will distort less than thinner mats) (Lord, 1981). Similarly, electronic force or pressure sensors may be affected by spatial distortion of the materials used to construct the sensor. Urry (1999) describes the ideal force sensor as a device that will respond the same to two equal forces regardless of the point of application or the area over which the force is applied on the sensor. Usually, to meet these conditions the contact surface of the sensors is made of a material with a high modulus of elasticity (for example, metal or ceramic). However, in cases were the contact surface of the sensor is made of a flexible material (for example, rubber or plastic) the contact surface may distort under load giving unpredictable results. The ideal electronic force sensors will give an output inversely proportional to the area of the sensor (Urry, 1999).

When measuring plantar foot pressure a temporal distortion may also occur (that is, the time elapsed between the application of pressure at the top of the platform system and its transmission through the mat to the lower surface). The nature of the time lapse is dependant on the visco-elastic properties of the materials used in the construction of the platform system and is particularly disruptive in gait studies, especially where these mechanically induced effects may add to the delays introduced by the electrical transduction of the pressure system (Lord, 1981).

Precise identification of anatomical sites in the plantar foot pressure map, especially in grossly deformed feet, is not possible and both foot-to-ground and in-shoe systems present with similar accuracy problems when trying to match plantar foot pressure on sites of interest to their correct anatomical location (Lord, 1981).

When the soft tissue of the foot makes contact with the external environment there are areas of low and high pressure, with higher pressure

over prominent bones and lower pressure radiating away from the bony prominence. If a small sensor is placed over the peak pressure site it will provide a good estimate of the real value. However, if a large sensor is placed over the same site, both covering areas of low and high-pressure, the pressure value of the larger sensor will be less than the smaller sensor (Urry, 1999). This occurs because pressure transducers used to quantify plantar foot pressure actually measure force, which is then converted to average pressure depending on the size of the sensor by the equation (Pressure = Force/Area) (Abboud *et al.,* 1996; Mueller, 1995). Thus, if the force is the same but the area differs, the sensor with the larger area will always produce a lower pressure value. Therefore, different systems will produce different average plantar foot pressure over the same anatomical site depending on the size of the pressure transducer (sensor) used. In addition, the type of pressure transducers used in different in-shoe systems vary (for example, capacitive, strain gauge, Force Sensing Resistor material, piezoelectric, etc.) thus the variability in accuracy, sensitivity, range, linearity and wear and tear, together with the variability in the sizes of sensors used by each in-shoe system makes comparability between studies using different systems not possible.

2.2.5 Conclusion

The study of plantar foot pressure has yielded a wealth of knowledge about the interaction between the foot and the external environment and the relationship of plantar foot pressure and lower limb pathologies. This review describes the development of systems to measure plantar foot pressure and how it has been possible, through the evolution of technology, to develop systems to measure foot-to-ground and in-shoe plantar foot pressure. More recently, systems that can also measure shear have been developed in the hope of broadening the study of how the external environment affects the lower limb. However, when studying tissue viability and how external pressure is related to the development of decubitus and neuropathic ulceration, research on the relationship between external pressure and ulceration alone is not sufficient to fully understand the process of tissue breakdown. In order to investigate the process of

ulceration further, reliable and valid methods of measuring the effects of external pressure on skin blood flow needs to be developed.

2.3 Methods to assess external pressure and skin blood flow

2.3.1 Introduction

During human walking the tissues in the plantar aspect of the foot are subjected to intermittent pressure. Evolution has adapted the soft tissues in the plantar aspect of the foot to cope with these "normal" insults to the skin's integrity. However, some studies have shown that in cases where peripheral neuropathy is present, high plantar foot pressure may lead to soft tissue breakdown and ulceration. As a result, over the years, research has concentrated on the effects of plantar foot pressure on foot function and integrity. Probably, because reliable and practical equipment has been readily available for quite a number of years. Indeed many devices exist that allows the study of plantar foot pressure. However, little is known of how plantar foot pressure interacts or causes damage to soft tissues. To understand how plantar foot pressure causes soft tissue breakdown it is vital to investigate the physiological-mechanical interactions between the skin and plantar foot pressure. With currently available technology, although in its infancy, it may now be possible to investigate the effects of plantar foot pressure on skin blood flow.

2.3.2 Devices to measure external pressure and skin blood flow

Various methods of investigating the effects of external pressure on the skin's microcirculation have been developed by a number of authors. However, most of the studies do not relate to the foot (Fromy *et al..,* 2000; Holloway *et al.,* 1976; Karlsmark *et al.,* 1987; Schubert *et al.,* 1994; Colin *et al.,* 1996; Schubert *et al.,* 1989; Abu-Own *et al.,* 1994; Yamaguchi *et al.,* 1991). And the few that relate to the foot have been unable to measure the effects of quantifiable plantar foot pressures on the skin's blood flow in the sole of the foot with the subject in a semi-weight bearing and/or weight bearing position (Cobb *et al.,* 2001; Abu-Own *et al.,* 1995; Abu-Own *et al.,*

1993; Mayrovitz *et al.*, 1997; Mayrovitz *et al.*, 1998; Meinders *et al.*, 1996) with the exception of Proano *et al.* (1992). It is therefore evident that a device capable of measuring the effects of quantifiable external pressures acting on the blood flow of the skin in the plantar aspect of the foot, with the subject in an upright position, would allow us to hypothesis about the possible effects of human walking on the skin's perfusion in the sole of the foot. Furthermore, being able to measure skin blood flow in both positions (that is, supine and upright) would allow us to investigate the effects of external pressure on the arterio-venous response.

Yamaguchi *et al.* (1991) developed a system to measure the effect of orthodontic brace forces on the peripheral circulation of the upper border of the gingival in the mouth using a laser Doppler fluxmeter fibre-optic probe fixed to a plate to hold the probe at 90° to the gingival. Pressure was applied to the teeth using springs that were hooked to buccal tubes bonded to the maxillary central incisors (see Figure 2-1).

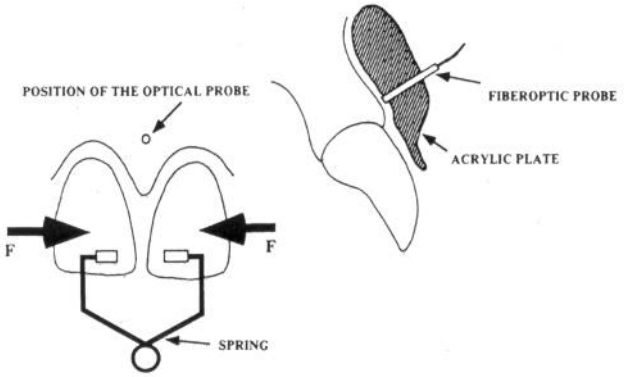


Figure 2-1: System developed by Yamaguchi *et al.* (1991) to measure the effects of orthodontic brace forces on gingival peripheral circulation.

Holloway *et al.* (1976) developed a system to measure the effects of external pressure loading on the microcirculation of the skin in the volar aspect of the forearm using ^{133}Xenon clearance. The system involved injecting 30 µCi ^{133}Xenon dissolved in 0.02 ml of sterile, pyrogen-free physiological saline into the skin using a 30-gauge needle and Hamilton syringe. This was followed by applying external pressure, using a servo-controlled loading device with an overall system frequency of 0.5 Hz, which

applied the load on a flat Plexiglas disc of 3 cm diameter centred on the site of injection.

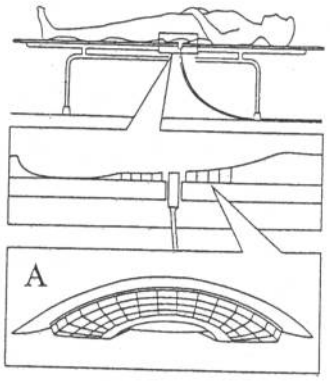


Figure 2-2: Device developed by Karlsmark and Kristensen (1987) to measure the effects of pressure-relieving materials on the sacral area using a laser Doppler fluxmeter to quantify skin blood flow.

Karlsmark and Kristensen (1987) designed a device to measure the effects of pressure-relieving materials in the prevention of pressure ulcers in the sacral area (see Figure 2-2). The device allowed for measurement of pressure on the sacral region when subjects lie in a supine position by placing a laser Doppler fluxmeter (LDF) probe (Perimed, Sweden) in small-bore holes in the polyacrylate board just below the central part of the sacral area. Following baseline recording, a circular closed cell foam plate (Comfeel PRD) with the centre removed was positioned under the sacral region. Through the hole, the LDF probe was placed for the second recording of cutaneous blood flow in the sacral region. Similarly, Schubert and Héraud (1994) developed a system to measure sacral skin microcirculation in elderly stroke patients in a supine and semi-recumbent position. The design consisted of an LDF probe (Perimed, Sweden), fixed to the skin on the sacral area using an angle-probe holder, which was embedded in a specially designed foam rubber pad covered with terry cloth to ensure that the probe did not influence skin pressure. Noble *et al.* (2003) also developed a system to measure the effects of pressure on skin blood flow in smokers and non-smokers in the sacrum. Colin and Saumet (1996) further developed a different device to measure skin blood flow and transcutaneous oxygen tension (tcpO2) on the sacral area (see Figure 2-3). This device consisted of an easily adjustable, height and length, mobile

arm. At the end of the arm, pressure on the probe holder was controlled by the amount of compressed air in the air chamber. Below the air chamber a strain gauge (Scaime, Annemasse, France) measured the pressure applied over the sacral area. The probe holder, below the strain gauge, made skin contact with the sacral area and contained both the LDF probe (Perimed, Sweden) and the $tcpO_2$ (Kontron Instruments, Watford, UK) probes.

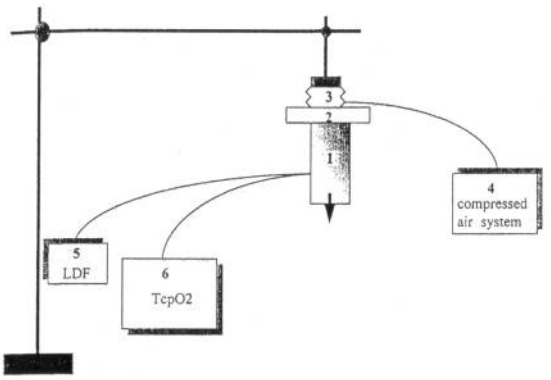


Figure 2-3: System developed by Colin and Saumet (1996) to measure skin blood flow and transcutaneous oxygen tension (tcpO2) on the sacral area. 1 - Probe holder for the laser Doppler fluxmeter and transcutaneous oxygen tension probes. 2 - Strain gauge. 3 - Air chamber. 5 - Laser Doppler fluxmeter. 6 - Device to measure transcutaneous oxygen tension.

Xakellis *et al.,* (1993) developed a system to measure dermal blood flow responses to constant pressure in a supine position in healthy old and young participants. The system consisted of a laser Doppler fluxmeter (Laserflo blood flow perfusion monitor, model BPM 403A; TSI, St. Paul MN) with a metallic probe (8.0 mm in diameter and 2.5 mm in thickness) that was connected via a fibre optic cable to a central unit. The probe was stuck to the left greater trochanter and subject was asked to lie on their side. External pressure was applied by means of an inflatable air-mattress overlay (Softcare, Gaymar Industries) that maintained an interface pressure below 32 mmHg (that is, approximately 4 kPa). Following the skin blood flow measurement the sensor was removed and a 1 by 2 inch inflatable bladder connected to an electric air pump and manometer (Gaymar Pressure Sensor Gauge, Orchard Park, New York) was placed below the left greater trochanter to measure the interface pressure (Xakellis *et al.,* 1993). Such method is not only time consuming but does not allow for synchronised recording of skin blood flow and external pressure and due to

removing the probe and replacing it with a bladder, the pressure at the site without the laser Doppler fluxmeter probe may be lower than with the probe. In another study, Schubert and Fagrell (1989) developed a device to measure the effects of pressure on skin blood flow on the sacral and Gluteus Maximus area (see Figure 2-4). The device consisted of a pivoted arm with a plexiglass head with a pressure cup containing an LDF probe fitted through a hole in the centre. The plexiglass head with a pressure cup was counter-balance with weight on the opposite site of the arm. Pressure was applied on the head by a sliding weight and skin blood flow was recorded using the LDF. Pressure was recorded using a thin pressure sensor (Kyowa Electronic Instruments Co. Ltd., Tokyo, Japan) and temperature with a thermistor[4] probe connected to a digital display (MC 8700, Exacon, Copenhagen, Denmark). Both probes were located under the plexiglass bottom plate.

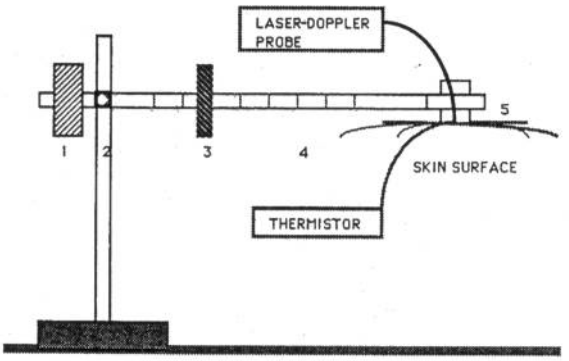


Figure 2-4: Device developed by Schubert and Fagrell (1989) to measure the effects of pressure on skin blood flow on the sacral and Gluteus Maximus area. 1 - Counter balance weight. 2 - Stand. 3 - Sliding weight. 4 - Balancing arm. 5 - Transucers.

Fromy *et al.* (2000) developed a device to apply progressive calibrated pressure to the skin of the finger (see Figure 2-5). The device is similar to that developed by Abu-Own *et al.* (1995) in that it uses a pivoted arm. With the subject in a supine position the laser Doppler probe (PF408, Periflux; Perimed, Järfälla, Sweden) is attached to one end of the pivoting arm. Next to the laser Doppler probe a plastic container is attached with one end of a polyethylene capillary tube secured in the container and the other to a

[4] A thermistor has a slow response rate that can take several seconds compared to a thermo-couple which is much faster. A thermo-couple to record temperature could have been more appropriate since the laser Doppler fluxmeter and pressure sensor would be sampling at a faster rate.

syringe filled with water in an automated syringe pump. Weights are attached to the other end of the pivoted arm to counter balance the laser Doppler probe on the opposite site. With the subject in a supine position and the finger resting on a block with the laser Doppler probe counter-balanced and just making skin contact, the automated syringe pump is started. As the plastic container fills with water progressive pressure is applied to the skin.

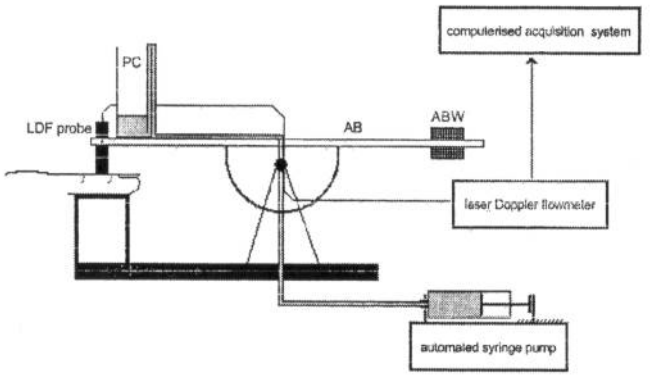


Figure 2-5: Device developed by Fromy *et al.* (2000) using a pivoted arm to measure the effects of pressure on skin blood flow in the finger.

Other authors have investigated the effects of externally applied pressure on the partial pressure of oxygen at the skin surface to determine if external pressure reduces oxygen levels in the cutaneous circulation over sites with and without bony prominences (Newson *et al.,* 1981). Using a "Hellige Servomed Oxymonitor SM261" mounted on a developed spring device, quantifiable pressure was applied to the skin and partial pressure of oxygen at the skin surface (P_sO_2) was recorded every 4 minutes when incremented pressure was applied. The spring device was able to apply a pressure of 0.67 kPa per millimetre of displacement (Newson *et al.,* 1981). However, with such system pressure may only be visually synchronised with the partial pressure of oxygen device or other systems (for example, the laser Doppler fluxmeter).

Studies investigating the effects of plantar foot pressure on skin blood flow are few but are leading the way towards the development of such equipment (Santos *et al.,* 2002; Cobb *et al.,* 2001; Mayrovitz *et al.,* 1998; Mayrovitz *et al.,* 1997; Meinders *et al.,* 1996; Abu-Own *et al.,* 1995; Abu-

Own *et al.,* 1993; Proano *et al.,* 1992). All these studies may be classified according to the type of equipment used to measure skin blood flow, which includes fluorescein flowmetry, laser Doppler imaging, and laser Doppler fluxmetry.

2.3.3 Fluorescein flowmetry and external pressure

Fluorescein flowmetry is an invasive method requiring the subject to drink 15 ml of 50% alcohol. This is followed by a bolus injection of 7 mg of sodium fluorescein per kilogram of body weight in 10 ml of isotonic saline injected into a cubital vein. Images are then taken of the subject's soles and blood flow is expressed as an index between the maximum fluorescence obtained between the first passage of sodium fluorescein through the circulation and the rise time. This is defined as the first interval of time between 10 and 90% of maximum fluorescein (Proano *et al.,* 1992; Perbeck *et al.,* 1985).

Proano *et al.* (1992) developed a system to measure plantar foot pressures using one of the EMED Gait Analysis Systems (Novel GmhH, Munchen, Germany) followed by measuring supine, standing and walking skin blood flow using fluorescein flowmetry. The standing and walking fluorescein flowmetry were carried out with the subjects standing on a podoscope and pictures taken using a Hasselblad electric motor-driven camera 500 EL/M (Hasselblad, Gothenburg, Sweden). However, one of the limitations of the study was that plantar foot pressures and plantar circulation were not measured simultaneously. Also, during the walking measurements the subjects were trained to stop on the podoscope and lifting the opposite foot for some of the pictures to be taken. Leading to a one legged standing position rather than a true walking position. Finally, this method uses invasive procedures requiring the subject to drink 15 ml of 50% alcohol followed by a bolus injection of 7 mg of sodium fluorescein per kilogram of body weight in a 10 ml of isotonic saline injected into a cubital vein. Thus, the practicalities and ethics of such procedure are questionable, especially if one wants to study a high-risk group.

2.3.4 Laser Doppler imager and external pressure

Laser Doppler imaging is a non-invasive method to measure skin blood flow with the device making no skin contact and is ideal to measure skin blood flow on skin grafts and ulcers. The system works on the principle of laser Doppler wave shift principle. The low power laser is used to scan an area on the skin and is controlled by an optical scanner consisting of two mirrors. At each measurement the laser penetrates to a depth of a few hundred micrometers and reflected back to a photo-sensor positioned in the scanner head. The laser light, where it interacts with moving cells, is spectrally broadened (termed the Doppler effect). This is converted into an electrical signal and stored in the computer as a product of blood cell speed and concentration (that is, flux). During each measurement procedure the captured perfusion value is colour coded. At the end of a scan a colour coded image representing blood flow is shown (Warden *et al.,* 1995). However a scan of an area 12 cm by 12 cm with a maximum of 4096 measurement sites takes approximately 4.5 minutes (Warden *et al.,* 1995). However, this is more the case of the LISCA systems which samples at 10 ms/pixel compared to the faster Moor laser Doppler imagers (Moor Instruments, Axminster, UK) which samples at 4, 10 and 50 ms/pixel (Mack, 1998). However, even the Moor laser Doppler imagers would be slow to capture the dynamic effects of plantar foot pressure on skin blood flow during walking since they are unable to capture the full area of the plantar aspect of the foot within a couple of seconds, thus its application in dynamic ambulation is questionable.

Mayrovitz *et al.* (1997) using the laser Doppler imaging (LDI) system (LISCA Development AB, Linkoping, Sweden) was able to measure heel blood perfusion responses to pressure loading and unloading in women. Subjects were placed in a supine position with their feet extended over the edge of the examination table. A baseline LDI scan of the plantar aspect of both heels was carried out at a distance of 19 cm. External pressure was applied to the right heel, for a period of 40 minutes, using a clear plastic plate 3 mm thick and a second scan taken (a limitation of this study is that the externally applied pressure was not quantified and may have been different between

subjects). During this period scans were taken of the off-loaded left heel. After the right foot was off-loaded scans were taken of the heel. At the end of each experiment scans of background calibration white card (Gray Card, Eastman Kodak Company, Rochester, New York) were taken with and without the plastic plate interposed to offset the calibration value which was subtracted from the raw data collected to account for any effects associated with light transmission through the plastic plate. Mayrovitz and Smith (1998) used the same technique developed by Mayrovitz *et al.* (1997) (already explained above) to study heel skin microvascular blood perfusion responses to sustained pressure loading and unloading in the supine position.

2.3.5 Laser Doppler fluxmeter and external pressure

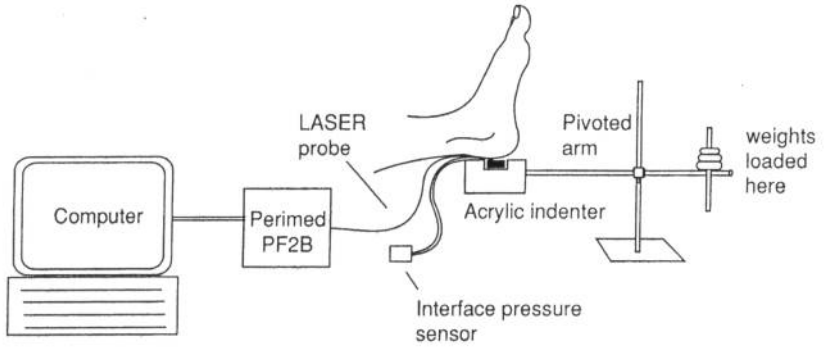


Figure 2-6: System developed by Abu-Own *et al.* (1995) to measure the effects of quantifiable external pressure on the posterior aspect of the calcaneus with the subject in a supine position.

Abu-Own *et al.* (1995) developed a method to measure the effects of quantifiable external pressure on the posterior aspect of the calcaneus with the subject in a supine position using a pivoted arm mounted on a stand and the Perimed PF 2B laser Doppler flowmeter (Perimed, Stockholm, Sweden) (see Figure 2-6). On one end of the pivoted arm a 5 cm diameter pressure-applying acrylic indenter housed the low profile laser Doppler probe and an interface pressure sensor (Talley Group Ltd, Romsey, Hants, UK). With the laser Doppler probe making skin contact forces of 50g (0.5 N) to 1500g (14.7 N) were applied to the skin on the heel region, using a cantilever mechanism with a central pivot, and loading weights on the opposite end of the arm. The device was designed to measure the effects of

compression on the heel's microcirculation in different hospital beds. In another study Abu-Own *et al.* (1993) developed a system to measure the effects of intermittent pneumatic compression of the foot on the microcirculatory function in arterial disease. The device consisted of an impulse foot pump (A-V Impulse System, Novamedix Ltd., UK), used to activate the foot muscle pump, which was fitted to a purpose-made slipper and made contact with the plantar aspect of the foot. Inflation and deflation applied intermittent compression to the foot. A LDF probe and a $tcPO_2$ probe, attached to the pulp of the big toe and dorsum of the foot respectively, measured the skin's microcirculatory function and transcutaneous oxygen tension. The device was unable to measure the direct effects of external pressure on the microcirculation of the foot. Silver-Thorn (2002) developed a system to measure the effects of force on tissue perfusion on the posterior aspect of the calf using a laser Doppler probe incorporated into a modified tissue indentor piston.

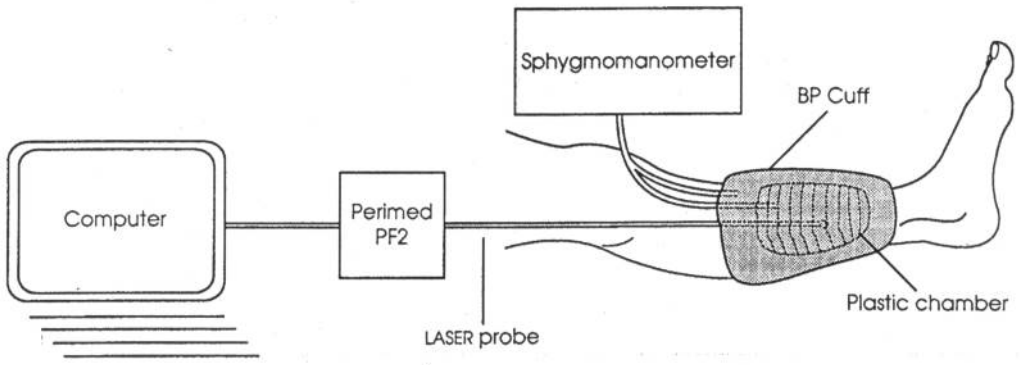


Figure 2-7: System developed by Abu-Own *et al.* (1994) to measure the effects of leg compression on skin blood flow in patients with chronic venous insufficiency.

Abu-Own *et al.* (1994) used a laser Doppler fluxmeter (LDF) probe, fitted in a polyethylene chamber and applied to the supramalleolar region underneath a blood pressure cuff, to measure the effects of leg compression in patients with chronic venous insufficiency (see Figure 2-7). The LDF probe was fitted 8 cm above the medial malleolus. The polyethylene chamber and the blood pressure cuff were connected together via a Y junction to ensure that the reading on the sphygmomanometer

reflected the pressure being exerted on the skin below the polyethylene chamber.

Meinders *et al.* (1996) developed a device to measure the microcirculation of the centre of the plantar aspect of the heel under quantifiable external pressure with the subject in a supine position. A shoe with a hole in the centre of the heel was attached to a rigid board at the end of the bed. A screw piston with a load cell (HBM load cell, type Z8) 2 cm in diameter and a laser Doppler Fluxmeter (LDF) probe (Applied Laser Technology, Maarheeze, The Netherlands) was inserted into the hole in the heel of the shoe. The subject's foot was placed in the shoe with the LDF probe making skin contact. Quantifiable external pressure was applied by turning the piston. Recording of pressure and blood perfusion was carried out using a computer. However, this system was only visually synchronised and only capable of taking supine measurements. Since human walking involves an upright posture and differences in skin blood flow exists between the patient in a supine and upright positions, due to the veno-arterial response causing vascular constriction when standing, the value of taking supine plantar foot pressure measurements is very limited.

Cobb and Claremont (2001) developed a dynamic in-shoe laser Doppler fluxmetry system to measure the effects of plantar foot pressure on skin blood flow over the metatarsal head area in diabetics. The system consisted of a laser Doppler sensor incorporated into a shoe with a pressure sensor. The pressure sensor was used as a pressure switch, enabling analysis of the loaded and unloaded foot. A mobile recording box enabled data collection without the use of trailing cables, with data downloaded to a computer at a later time for analysis. Although plantar foot pressure was not quantified, results showed that following loading an almost linear increase in skin perfusion blood flux occurred during the swing phase. The short time phase of loading in normal subjects was not sufficient to induce a hyperaemic response. However, in periods of prolonged weight bearing (for example when standing for two minutes) followed by non-weight bearing, a hyperaemic response was induced (Cobb *et al.,* 2001).

Santos *et al.* (2002) developed a system, similar to Meinders *et al.* (1996), using a pressure sensor type LM-50KA (Kyowa Electronic Instruments Co., Ltd, Tokyo, Japan) capable of measuring the effects of quantifiable plantar foot pressure on skin blood flow in the centre of the heel in an upright position using an integrated pressure-blood flow piston mechanism housed in a medical-surgical shoe (Darco International Inc, USA). The system has now been modified to use two widely available systems allowing for minimum equipment development and accessibility of this type of equipment for future research. The laser Doppler fluxmeter DRT4 (Moor Instruments Ltd, UK.) has been integrated with the Pedar standard (Novel GmhH, Munchen, Germany) using a standalone pressure capacitive sensor. The devices are electronically synchronised in real time using a specially developed midway synchronisation box. The advantages of this system are that both quantifiable plantar foot pressure and duration of pressure application can be controlled when investigating the effects of plantar foot pressure on skin blood flow in an upright position. However, this equipment is not capable of measuring the effects of dynamic plantar foot pressure on skin blood flow.

2.3.6 Conclusion

Research in the foot has mainly concentrated on plantar foot pressure, with high plantar foot pressure correlated with neuropathic ulceration. These studies have suggested a mechanical interaction between plantar foot pressure and tissue viability, however the mechano-physiological processes may not be investigated using pressure systems alone. This review suggests that with current technology the physiological responses of skin blood flow to mechanical plantar foot pressure should be investigated in more detail, both during static and dynamic loading, and more work needs to be done to perfect these techniques and make such equipment(s) widely available.

CHAPTER 3

ANATOMY, PHYSIOLOGY AND TISSUE VIABILITY

3. CHAPTER 3: Anatomy, physiology and tissue viability

3.1 Introduction

It is thought that the main functions of the human foot are to support body weight and serve as a lever to propel the body forwards during locomotion (Snell, 1981[b]). During ambulation the plantar aspect of the heel is one of the weight-bearing areas of the human foot. Through evolution the glaborous skin and subcutaneous structures of the heel have adapted to endure the rigors of human dynamic activity. Maintaining homeostasis within the living tissues beneath the heel involves precise interaction within various systems. In some cases failure by some systems may be compensated by other systems; in other cases complete failure leads to damage, soft tissue breakdown and ulceration. Thus, understanding the anatomy and physiology is vital, before investigation how the skin reacts under external pressure and comment on how tissue viability may be compromised.

3.2 Blood supply to the heel

The smallest arteries are called arterioles (less than 1 mm in diameter). Arterioles form an intricate branch structure with a complex framework of anastomoses between them. Most superficial arterioles connect with venules through capillary vessels where vessel to tissue to vessel exchange occurs. The venules connect with veins and return blood back to the heart (Gush *et al.,* 1984; Snell, 1981[a]).

In the deep dermis small branches of subcutaneous branches freely anastomose in a plane horizontal to the skin surface and is referred to as the cutaneous arterial plexus (Sparks, 1978). The calcaneal anastomoses is supplied both, by the peroneal artery and lateral plantar artery (Romanes, 1999). Branches from this cutaneous deep plexus supply the deep sections of sweat glands. In addition, arterioles arise obliquely through the dermis from the deep plexus (Sparks, 1978).

3.3 Microcirculation of the skin

As the arterioles extend from the cutaneous arterial plexus their smooth muscle coat gets thinner (Sparks, 1978). Just below the dermal papillae the arterioles of almost microscopic size (approximately 100 μm or less) and metarterioles form a horizontal anastomatic network called the subpapillary arterial plexus. Metarterioles and capillaries ascent to the dermal papillae and bring blood close to the epidermal basal cells (Seeley *et al.,* 2000[c]; Young *et al.,* 2000[b]; Tortora *et al.,* 1993[c]; Leeson *et al.,* 1985[b]; Gush *et al.,* 1984; Sparks, 1978). The capillary loops turn round and drain into small venules. The small venules intermingle with the subpapillary arteriolar plexus forming a much bigger subpapillary venous plexus. Arteriovenous anastomoses connect arterioles and venules in the subpapillary plexus (Sparks, 1978).

Proximal to the originating artery, the diameter of the arteriole is at its widest and is composed of a tunica interna (inner most made up of endothelium covered with a basement membrane and internal elastic lamina), tunica media (consisting of smooth muscle with a few elastic fibres) and finally, tunica externa (made of mostly elastic and collagen fibres). The arteriole is at it narrowest at the most distal end where it joins a metarteriole near the capillaries and the tunics consist of an endothelial layer surrounded by a few scattered muscle fibres (Seeley *et al.,* 2000[c]; Young *et al.,* 2000[b]; Tortora *et al.,* 1993[c]; Leeson *et al.,* 1985[b]). At some places arteriovenous anastomoses may emerge from an arteriole, bypass the capillaries and drain straight into a venule. In some places metarterioles flow straight through the capillary network via thoroughfare channels and drain into venules. (Cragg, 2000[a]; Seeley *et al.,* 2000[c]; Tortora *et al.,* 1993[c]; Junquiera *et al.,* 1992[b]).

Arteriovenous anastomoses are numerous in the glaborous thick skin of hands and feet (Junquiera *et al.,* 1992[b]; Castronuovo *et al.,* 1987; Leeson *et al.,* 1985[b]; Sparks, 1978; Grant *et al.,* 1931). The arteriovenous anastomosis regulates blood pressure, blood flow, temperature and local conservation of heat by vasodilatation or vasoconstriction (Junquiera *et al.,*

1992[b]; Castronuovo *et al.,* 1987; Leeson *et al.,* 1985[b]; Engelhart *et al.,* 1983; Sparks, 1978; Grant *et al.,* 1931). When vessels in the arteriovenous anastomoses contract, all blood is forced to circulate within the capillary network thus maximally increasing nutritional exchange. The arteriovenous anastomosis appears to be mainly controlled by nerves and are richly innervated by the sympathetic and parasympathetic nervous systems (Junquiera *et al.,* 1992[b]; Leeson *et al.,* 1985[b]; Sparks, 1978). The arteriovenous anastomoses do not contribute to the nutrition of the tissues (Engelhart *et al.,* 1983).

The capillaries, abundant within the dermal papillae at the epidermal/dermal junction, are microscopic vessels (3 to 9 μm in diameter and generally approximately 0.2 to 1 mm long) originate from metarterioles and empty into venules (Mehler, 2000; Young *et al.,* 2000[b]; Junquiera *et al.,* 1992[b]; Leeson *et al.,* 1985[b]; Zweifach, 1971). They consist of a thin endothelial layer of cells surrounded by a base membrane. Near the metarteriole, a pre-capillary sphincter (a smooth muscle fibre surrounding the capillary) regulates capillary blood flow. The prime function of the capillaries is to allow the exchange of nutrients and waste products between the blood and tissue cells (Seeley *et al.,* 2000[c]; Tortora *et al.,* 1993[c]; Junquiera *et al.,* 1992[c]). The capillaries are well suited for this task due to the thin nature of their walls as blood and tissue substances only have to squeeze through one layer of cells. In addition, they form extensive branching networks between them, increasing surface area and allowing the rapid diffusion and filtration of large quantities of materials (Seeley *et al.,* 2000[c]; Tortora *et al.,* 1993[c]).

Venules collect the deoxygenated blood from capillaries and drain into veins. Most proximal to the capillaries the venules consist of an endothelial tunica intima and a tunica externa of connective tissue. As the venules approach the veins, they also develop a tunica media. The tunica intima and media is thinner in veins than in arteries but the tunica externa is much thicker (Seeley *et al.,* 2000[c]; Tortora *et al.,* 1993[c]).

3.4 Regulation of skin blood flow

Cutaneous arterioles and arteriovenous anastomoses are controlled by factors released by the endothelium, the autonomic nervous system, and local metabolic substances but not by myogenic autoregulation (Cragg, 2000[a]; Cragg, 2000[b]; Luscher *et al.,* 1997; Johnson, 1978). The endothelial cells, which provide the inner lining of all blood vessels, provide control of blood flow to the tissues by releasing humoral factors that have the effect of vasoconstriction or vasodilation. Shear forces, neurotransmitters, hormones and substances derived from platelets stimulate endothelial cells to release nitric oxide, a muscle relaxing factor that causes vasodilatation and increases blood flow. In comparison, the release of endothelin-1 by endothelial cells has the effect of vasoconstriction and a decrease in blood flow. Thus, endothelial cells have a vital role in the homostatis of skin blood flow (Luscher *et al.,* 1997).

The main role of arterioles is to regulate skin blood flow through capillaries (Seeley *et al.,* 2000[c]). When the arteriolar smooth muscle contracts vasoconstriction decreases skin blood flow through capillaries. Conversely, when the smooth muscle surrounding the arterioles relaxes vasodilatation increases blood flow through the capillary network (Applegate, 2000[c]; Tortora *et al.,* 1993[c]; Junquiera *et al.,* 1992[b]). In addition, thoroughfare channels and capillary sphincters also play a role in the regulation of blood flow through the capillary bed (Applegate, 2000[c]; Seeley *et al.,* 2000[c]; Tortora *et al.,* 1993[c]). Thoroughfare channels and pre-capillary sphincters are controlled locally depending on the metabolic needs of the tissues. (Seeley *et al.,* 2000[c]; Junquiera *et al.,* 1992[b]; Johnson, 1978). As the rate of tissue metabolism increases chemical vasodilatation substances increase in concentration within extra-cellular fluid (for example, carbon dioxide, lactic acid, etc.) and have the effect of relaxing the smooth muscle of pre-capillary sphincters, thoroughfare channels and arterioles aiding the removal of tissue metabolites. However, tissue ischaemia also causes a similar response with vasodilatation of vessels. The vasodilatation induced increase in blood flow continues until all the metabolism by-products are reduced in concentration and the nutrient supply to the smooth muscle of

the pre-capillary sphincters are replenished. When this occurs, the pre-capillary sphincter vasoconstricts until there is another build up in the concentration of by-products or nutrient deficiency (Applegate, 2000[c]; Seeley *et al.,* 2000[c]; Johnson, 1978).

The proximal section of thoroughfare channel (that is, the metarteriole) is surrounded by scattered smooth muscle fibres, which regulate blood flow by contracting or relaxing. Most distally, as the thoroughfare channel approaches the venules, they have no such smooth muscle fibres. The role of the thoroughfare channel is as a low resistance pathway that opens when the pre-capillary sphincters reduce blood flow through the capillary network and prevent a built up of pressure at the arteriole side (Tortora *et al.,* 1993[c]).

Pre-capillary sphincters regulate blood flow through the capillaries. Skin blood flow through capillaries is not continuous but intermittent. This occurs as a result of the smooth muscle within the arteries, arterioles, thoroughfare channel and the pre-capillary sphincters contracting and relaxing intermittently and is termed vasomotion (Applegate, 2000[c]; Seeley *et al.,* 2000[c]; Tortora *et al.,* 1993[c]).

3.5 Blood flow velocity

Blood flow is the volume of blood that flows through any tissue in a specific period of time (Applegate, 2000[c]; Tortora *et al.,* 1993[c]). Local blood flow is proportional to the metabolic needs of the tissue, increasing to supply the increased requirement of oxygen and nutrients and remove any build up of metabolites (Seeley *et al.,* 2000[c]). In skin, blood flow is also related to thermoregulation (Seeley *et al.,* 2000[c]). The velocity of blood flow is inversely related to the total cross-sectional area of blood vessels. Thus the velocity of blood decreases as it flows from arteries to arterioles to capillaries and increases as it flows from the capillaries to venules to veins (Applegate, 2000[c]; Tortora *et al.,* 1993[c]). In an adult, the average cross-sectional area of the aorta is 3 to 5 cm^2 with a mean blood velocity of 40 cm/sec. Within capillaries the average cross sectional area is about 4500 to 6000 cm^2 with a mean blood flow velocity of 0.1 cm/sec and within the

venae cavae the cross sectional area is about 14 cm^2 with a velocity of 5 to 20 cm/sec (Tortora *et al.*, 1993[c]).

Microangiopathy appears to cause not only damage to the walls of small blood vessels supplying the skin but also a disturbed microcirculation flow pattern. Abu-Own *et al.* (1994) found that skin blood flow of lipodermosclerotic tissue was increased when compared to healthy controls suggesting that the coiled and distended blood vessels observed by other authors using microscopy may account for the differences in blood flow between the two groups, since no significant differences in haematocrit levels between the two groups was observed.

3.6 Nutritional exchange

Nutritional exchange occurs at capillary level due to diffusion, vesicular transport and bulk flow. The most important type of exchange is diffusion (that is, the movement of certain substances across the capillary wall from higher concentrations to lower concentrations), which allows solute exchange and the movement of oxygen, carbon dioxide, glucose, amino acids, hormones, etc., and provides the bulk of the tissues' nutritional exchange (Applegate, 2000[c]; Tortora *et al.*, 1993[c]; Intaglietta *et al.*, 1978; Zweifach, 1971). Vesicular transport allows for a small quantity of large, lipid insoluble molecules to travel across the capillary wall (Tortora *et al.*, 1993[c]; Intaglietta *et al.*, 1978). Finally, the second most important as it allows the regulation of the relative volumes of blood and interstitial fluid is bulk flow that occurs due to differences between capillary and tissue pressure. The high hydrostatic capillary pressure and low osmotic capillary pressure near the arterial end allows for fluid transport from within capillaries to the surrounding interstitial spaces or tissues (Applegate, 2000[c]; Mehler, 2000; Tortora *et al.*, 1993[c]; Junqueira *et al.*, 1992[a]; Intaglietta *et al.*, 1978; Zweifach, 1971; Kitchin, 1963). As vessel fluid exits the capillary the hydrostatic capillary pressure decreases towards the capillary venous end and the osmotic capillary pressure increases due to a build up in concentration of proteins within the capillaries and lower hydrostatic capillary pressure (Applegate, 2000[c]; Mehler, 2000; Junqueira *et al.*, 1992[a];

Intaglietta *et al.,* 1978; Kitchin, 1963). As a result of the lower osmotic capillary pressure fluid is forced from the surrounding interstitial spaces into the capillaries near the venous end (Mehler, 2000; Tortora *et al.,* 1993[c]; Junqueira *et al.,* 1992[a]; Intaglietta *et al.,* 1978). Thus, where sufficient diffusion to meet the needs of the local soft tissues is affected (for example, by applying external pressure, and reducing or arresting blood flow and the transport of nutrients to soft tissues) slow or fast cellular death may occur leading to soft tissue breakdown and ulceration.

When blood flow to the skin is temporarily arrested, restoration of blood flow is accompanied by a large increase in supply (reactive hyperaemic response) proportional to the level of tissue ischaemia (that is, the duration of circulatory arrest and the metabolic rate of the tissues at that time) suggesting that a local vasodilator is released in proportion to the difference between the supply of blood and tissue metabolism (Cragg, 2000[a]; Johnson, 1978; Sparks, 1978). Antihistamine does not affect the reactive hyperaemic response of tissue (Sparks, 1978). The reactive hyperaemic response is mediated by the local accumulation of vasodilatation metabolites during the circulatory obstruction because studies have shown reactive hyperaemia in both sympathetically and somatically denervated skin, although lack of oxygen also plays a significant role (Cragg, 2000[a]; Johnson, 1978; Sparks, 1978; Carrier *et al.,* 1964; Ross *et al.,* 1962; Crawford *et al.,* 1959). However, the reactive hyperaemic response has also been observed with high levels of blood oxygen present. Khan *et al.* (1991) found that following breathing 100% oxygen at 1 and 2 atmospheres absolute in a hyperbaric chamber the hyperaemic response was still present after arterial occlusion, with recovery times to baseline significantly improved. This was probably due to a reduction in the vasodilators produced during the period of hypoxia as a result of higher levels of oxygen present in the local tissues. In addition, the high levels of oxygen also had a small direct vasoconstrictor effect.

3.7 Lymphatic supply to the skin

The lymphatic system allows draining of interstitial fluid, transport of dietary fats and provides immune responses (Tortora *et al.,* 1993[d]). Since more fluid leaves the capillaries than is reabsorbed through bulk flow, the lymphatic capillaries drain any excessive build-up of soft tissue fluid. Thus, at the most superficial level lymph capillaries provide a fine network of vessels that drain lymph from the tissues in the dermis and hypodermis into the lymphatic system (Seeley *et al.,* 2000[c]; Tortora *et al.,* 1993[d]; Junqueira *et al.,* 1992[a]; Snell, 1981[a]). The lymphatic capillaries are slightly larger than blood capillaries, lack a base membrane and the cells within the vessel wall overlap slightly and are loser (Seeley *et al.,* 2000[c]; Tortora *et al.,* 1993[d]). This unique structure of the vessel walls allows for interstitial fluid to only flow into the vessel and not out. The wall of the lymphatic capillaries is sensitive to differences in pressure between interstitial fluid outside and lymph fluid inside the vessel. When tissue fluid pressure is highest (that is, when tissues are compressed due to external pressure or fluid tension) the cells within the vessel wall open up by contraction of the endothelial cells and allow tissue fluid to flow into the lymphatic capillary. When tissue fluid pressure is lowest, the gaps within the vessel wall close (Seeley *et al.,* 2000[c]; Tortora *et al.,* 1993[d]; Junquiera *et al.,* 1992[b]). Thus, human locomotion may have a direct effect on capillary exchange. During the stance phase the tissues in the sole of the foot are compressed by external pressure. This although it may reduce or arrest capillary blood flow (reducing or arresting the supply of vital oxygen to tissue cells), it may assist in absorbing interstitial fluid into the blood and lymphatic capillaries by raising interstitial tissue pressure. During the swing phase the quick reduction in interstitial pressure as a result of removing external pressure causes a slight build-up of capillary blood pressure at the arterial end and may assist in promoting a fast transport of nutrients to the extra-vascular cells allowing for post-starvation tissue recovery.

The lymph fluid travels up through lymph nodes and back into the blood stream at the root of the neck by the right lymphatic duct and the thoracic duct (Tortora *et al.,* 1993[d]; Snell, 1981[a]; Junquiera *et al.,* 1992[b]).

3.8 Biomechanics of the skin

The epidermis appears to have a barrier function with the dermis determining the resistance to mechanical pressure through the viscoelastic properties of its extracellular macromolecules like collagen, polysaccharide glycosaminoglycans and elastin (Kernick *et al.,* 1999; Kabagambe *et al.,* 1994). The polysaccharide glycosaminoglycans forms a highly hydrated gel-like substance that allow fibrous proteins to embed themselves and the polysaccharide gel allows for the diffusion of nutrients and metabolites between the tissues and blood to occur (Kernick *et al.,* 1999). However, extracellular macromolecules reduce with age and the ability of the skin to withstand mechanical pressure reduces with a direct transfer of mechanical force to microvessels and thus, making pressure damage more likely. This is compounded by a reduction in the thickness of the fat pad (Kabagambe *et al.,* 1994).

A study by Robbins *et al.* (1993) found that immediately after the stimulation with high intensity vertical and horizontal stimulation all sites on both thin skin (on the thigh) and thick skin (on the plantar aspect of the heel) had observable small sites of maximum redness. Twenty-four hours after the stimulation no sites of redness or weeping lesions were observed at the heel but small sites of redness were still visible in the thin skin of all 12 subjects and three had developed weeping lesions at the site of maximum redness. This demonstrates the greater biomechanical resilience to trauma of thick glaborous skin than thin skin.

Tosti *et al.* (1977) used a ballistrometer to assess the plasto-elastic properties of the skin and although the foot and in particular the thick skin on the plantar aspect was not tested the results are of interest. A ballistrometer measures the "drop impact" of a body (metallic hammer) onto stationary skin. Where the stationary object is elastic the impacting object will rebound and conversely if the stationary body is inelastic the impacting object will adhere to the surface. The coefficient of restitution represents the ratio of the rebounding height divided by free falling height and may be expressed as a percentage of the rebound energy. In skin the coefficient of

restitution may provide information with regards to cutaneous tone. A study was carried out in 46 healthy subjects (age range 8 to 80) and twelve skin areas were tested per subject. In all sites the tested percentage of rebound energy degraded with age (Tosti *et al.,* 1977). Thus, it may be postulated that biomechanically, skin looses its elasticity with increasing age and may have an effect in the distribution of plantar foot pressure and its relation to skin blood flow in the aging foot. In tests carried out in pathogenic skin the results showed an increase in the coefficient of restitution with increasing water content of the skin ((Tosti *et al.,* 1977). This suggests that dry or calloused skin would reduce the skin's elastic properties and its ability to deform to irregularities in the ground/shoe (hence reducing the protective biomechanical properties of the skin to increase its surface area by engulfing any irregularities of the ground) in an attempt to reduce peak pressure during ambulation. The higher peak pressure in dry or calloused skin may have a greater effect on skin blood flow.

3.9 Optical qualities of the skin and blood

3.9.1 Optical qualities of the skin

The skin colour is derived from three pigments: melanin, carotene and haemoglobin but is also dependent on the thickness of the stratum corneum (Seeley *et al.,* 2000[a]; Hunter *et al.,* 1999[b]; Tortora *et al.,* 1993[a]; Leeson *et al.,* 1985[b]). Melanin is produced by melanocytes most superficially in the epidermis. The number of these cells between humans is about the same with differences in skin colour, from pale yellow to black, dependant on the amount of melanin produced by the cells (Applegate, 2000[a]; Hunter *et al.,* 1999[b]; Tortora *et al.,* 1993[a]; Leeson *et al.,* 1985[b]; Sparks, 1978). Carotene, a yellow-orange pigment, may be found in individuals of Asian ancestry within the stratum corneum and fatty areas within the dermis and subcutaneous layer (Applegate, 2000[a]; Hunter *et al.,* 1999[b]; Tortora *et al.,* 1993[a]). In Caucasians, the epidermis contains small amounts of melanin hence is more translucent. Thus, it appears pink to red depending on the amount of skin blood flow and oxygen content of haemoglobin flowing through the capillaries (Hunter *et al.,* 1999[b]; Tortora *et al.,* 1993[a]; Sparks, 1978). Abu-Own *et al.* (1994) reported that skin pigmentation appears to

have no direct effect on the laser Doppler flux readings however, hyperpigmentation may reduce the penetration of the laser light resulting in lower flux output.

Before reaching viable tissue the light has to pass through the stratum corneum, hence the thickness, composition and morphology of this layer will have a modifying factor. At near-normal incidence (that is, nearly perpendicular) a small level of refraction occurs between the air and stratum corneum equivalent to approximately 4 to 7% over the entire spectrum of light from 250 to 3000 nm for normal skin irrespective of colour. Because the surface of the epidermis is not smooth and planar (as it contains ridges formed by the dermal papillae) back-scattered light occurs because the skin's surface is not specular and because as it passes through the rough layer the light is diffused. A good example is comparing skin to rough translucent glass (that diffuses any emitted light). If smooth, polished transparent glass were used instead the light would travel through it. The epidermis and its stratum corneum are highly absorbent to wavelengths of less than 300 nm largely because of its peptide bonds and other substances, including melanin and its precursor tyrosine, tryptophan, nuclei acids and urocanic acid (Anderson *et al.,* 1981).

The optical qualities of the dermis are distinctly different to that of the epidermis due to the reflective properties of its structure and composition. The dermis may be described as a turbid matrix of soft tissue with the optical scattering of light inversely related to wavelength. Thus, optical penetration depth is related to the function of wavelength. Within the dermis the pigments of haemoglobin, oxy-haemoglobin, beta-carotene and bilirubin are the major absorbers of visible radiation (Anderson *et al.,* 1981).

Wavelength (nm)	Depth (μm)	Depth (mm)
250	2	0.002
280	1.5	0.0015
300	6	0.006
350	60	0.06
400	90	0.09
450	150	0.15
500	230	0.23
600	550	0.55
700	750	0.75
800	1200	1.2
1000	1600	1.6
1200	2200	2.2

Table 3-1: The approximate optical penetration depth in fair Caucasian skin (adapted from Anderson *et al.*, 1981).

Across the optical spectrum, different wavelengths (from ultraviolet light at 250 nm to infrared light at 3000 nm) will reach different depths within tissues (see Table 3-1) (Anderson *et al.*, 1981). This has been discussed in the previous chapter with regards to laser Doppler flowmetry. However, at 350 to 1200 nm melanin appears to be the major absorber of radiation within the epidermis, especially at the shorter wavelengths.

3.9.2 Optical qualities of the blood

The optical qualities of blood with regards to laser Doppler flowmetry are dependent on the movement of cells within vessels. Thus, it is important to understand the composition of blood and its cells. Whole blood contains 55% blood plasma and 45% are formed elements (that is, cells and cellular fragments) (Applegate, 2000[a]; Seeley *et al.*, 2000[b]; Tortora *et al.*, 1993[b]; Leeson *et al.*, 1985[a]). The blood plasma contains 91.5% water and 8.5% solutes of which most are plasma proteins. The formed cells are of three types: platelets (or thrombocytes), white blood cells (or leukocytes) and red blood cells (or erythrocytes) (Seeley *et al.*, 2000[b]; Tortora *et al.*, 1993[b]). Although some of the formed elements within the blood other than erythrocytes will contribute to the laser Doppler fluxmeter's signal, since red blood cells constitute up to 95 to 99% of all the formed elements, red blood cells will contribute to the majority of the signal generated by instruments. Thus, indirectly laser Doppler fluxmeter values may indicate nutritional transport to tissues.

3.10 Pathophysiology of the skin

3.10.1 Ischaemia of soft tissues

It is generally accepted that the underlying cause of ischaemia and necrosis of soft tissues is related to the effects of mechanical pressure, the effect of which is to restrict nutritive blood flow, leading to a reduction in nutrient supply, primarily oxygen and affecting waste product removal (Newson *et al.,* 1981). Skin blood flow may be reduced or arrested by vasoconstriction of arteries and arterioles in the deep and superficial plexus of the dermis or most superficially by collapse of the capillary walls in the dermal papillae (Ashton, 1975). Collapse of the capillaries or capillary closing pressure occurs when external pressure exceeds the mean capillary pressure of 32 mmHg (4.3 kPa) (Russell, 1998). In arterioles, the rate of blood flow is determined by the diameter of the vessel's internal wall. The vessel's internal wall is controlled by various forces; by the intravascular pressure (P_{IV}) pushing at right angles to and expanding the wall and the surrounding extra-vascular tissue pressure (P_T) opposing this. The difference between the two determines the total distending force of the vessel or transmural pressure (Ashton, 1975; Ashton, 1963). In addition, the distending force is balanced by the constricting force, due to the circumferential tension (T_C) of the vessel wall. The circumferential tension of the smooth muscle surrounding the vessels is composed of two components, the elastic (T_E) and active tension (T_A). The elastic tension consists of the elastic properties of the vessel wall (mainly by collagen and the elastic fibres but also by the relaxed smooth muscle fibres) and depends on the degree of stretch of the vessel wall (Ashton, 1975; Ashton, 1963; Burton, 1951). The active tension is mediated by the vasomotor or myogenic contraction of the smooth muscle surrounding the vessel (Ashton, 1975; Ashton, 1963; Burton, 1951; Bayliss, 1902). Stability of the vessel wall is maintained when the transmural pressure (P_{PT}) equals the circumferential tension (T_C) divided by the radius (r) of the vessel (that is, $P_{PT} = T_C/r$) (Ashton, 1975; Ashton, 1963; Burton, 1951).

During neurally mediated vasoconstriction the circumferential tension divided by the vessel radius becomes greater than the transmural pressure

hence the vessel's diameter reduces in size. In small arterioles, where the transmural pressure is low enough for the elastic tension to be zero (that is, when the elastic fibres decrease to their unstretched length) and the radius is also small, a small increment in circumferential tension from vasomotor tone is sufficient for T_C/r to be greater than the transmural pressure and the vessels to close. This is termed critical closing pressure. Thus, smaller arterial vessels will close sooner than bigger vessels (Ashton, 1975; Rodbard, 1971[a]; Ashton, 1962[b]; Ashton, 1963). In the finger and forearm, subjects with hypertension had a higher flow cessation pressure than "normals" (Ashton, 1963; Ashton, 1962[c]).

Applying the equation $P_{PT} = T_C/r$ to the effects of applying external pressure to the skin and its effects on arteries and arterioles within the dermis. When external pressure is applied to the skin the extra-vascular tissue pressure increases. This has the effect of reducing the transmural pressure below T_C/r and the vessel collapses or closes arresting skin blood flow. When the external pressure is excessive the enormous tissue pressure will result in a negative transmural pressure and complete collapse of the vessel structure. Beer (1971) using an *in vitro* model found that when transmural capillary pressure becomes zero or negative the vessel collapses as a direct result of increasing extra-vascular tissue pressure due to an increase in volume and pressure. The bigger the vessel and the nearer to the heart, the higher the intravascular pressure. The greater the intravascular pressure the higher the tissue pressure needed (in this case related to the application of external pressure) to reduce the transmural pressure of the vessel. So bigger vessels are more resilient at withstanding the effects of external pressure. Because smaller vessels are most superficial and bigger vessels deeper, when pressure is applied the most superficial vessels will close first. Subsequent closure of vessels is ranked depending on proximity to the peak pressure applied and diameter/vessel's transmural pressure. Also, the higher the external pressure the more tissue destruction there is going to be, as ischaemia will occur deeper into the dermis.

Capillaries have a thin vessel wall structure and are particularly vulnerable to changes in tissue pressure. They also have a lower intravascular pressure than arterioles thus, upon an increase in tissue pressure (for example, when external pressure is applied) capillary collapse and blood flow arrest through the nutritive capillaries is likely to occur before arteriole closure (Ashton, 1975). Fromy *et al.,* (2000) in a study investigating the effects of external pressure on skin blood flow in the finger reported that the mean decrease in laser Doppler flux occurred at an absolute pressure value close to that reported by Ashton (1975) and Russell (1998) for the capillary closing pressure. This suggests that direct mechanical compression of tissues is involved in reducing/arresting capillary nutritive blood supply to the skin. Furthermore, using a laser Doppler fluxmeter and a digital cuff connected to a Statham transducer, Fisher *et al.* (1995) reported skin perfusion pressure at the toe as 81.4 ± 18.8 mmHg (10.9 ± 2.5 kPa) for 11 healthy controls and 38.4 ± 21.7 mmHg (5.1 ± 2.9 kPa) for 14 patients with peripheral arterial occlusive disease. Castronuovo *et al.* (1987) found similar results in the plantar skin perfusion pressure of the toe with 73 ± 5 mmHg (9.7 ± 0.7 kPa) in 32 healthy limbs against 17 ± 3 mmHg (2.3 ± 0.4 kPa) in 26 ischaemic limbs. Such differences between healthy and patients with peripheral arterial occlusive disease suggest that the nutritional capillaries will close at a lower external pressure for the patients with peripheral arterial occlusive disease than for healthy controls. This may explain why high-risk patients with arterial disease may be more prone to mechanically mediated soft tissue breakdown as tissue ischaemia and cellular death through starvation may occur at lower pressures, especially in the presence of diminished pain sensation where the body's protective senses are absent. It is also important to note that dorsal skin perfusion pressure on the toe was lower for both healthy and ischaemic limbs than on plantar glaborous skin (55 ± 5 mmHg (7.3 ± 0.7 kPa) verses 16 ± 4 mmHg (2.1 ± 0.5 kPa) respectively) (Castronuovo *et al.,* 1987). This difference may be explained by the presence of numerous thermoregulatory arteriovenous anastomoses in plantar thick skin.

In animal *in vivo* studies following ischaemia the pressure area remained bloodless, except for the larger arteries. The sequence of opening of blood vessels following pressure ischaemia is as follows: the smaller blood vessel walls remained pressed together except for the larger arteries that were forced open by the higher transmural pressure. The opening of the larger veins followed this and finally, the capillary network opened and was re-perfused (Willms-Kretschmer *et al.,* 1969). Thus, the re-opening of capillaries, arterioles and venules appears to be related to an increase in intravascular pressure or decrease in tissue pressure having the effect of raising arterial transmural pressure (Ashton, 1975; Rodbard, 1971[a]; Ashton, 1962[a]; Willms-Kretschmer *et al.,* 1969). In the case of arterioles, re-opening may also occur without a change in transmural pressure but as a result of the action of low levels of local oxygen and the accumulation of local vasodilatator metabolites, however this mechanism is inadequate if the transmural pressure is lowered to a level much below the level at which flow ceases (Carrier *et al.,* 1964; Ashton, 1962[a]; Ross *et al.,* 1962; Crawford *et al.,* 1959). Thus, at low external/mechanical pressures to the soft tissues, very close to the arteriole closing pressure, an accumulation of local vasodilatating metabolites relaxes smooth muscle causing the elastic fibres to exert a resisting force springing the vessel to open.

The resulting post-ischaemic hyperaemic response is attributed to the metabolic dept caused by reduction/arrest of skin blood flow and appears to reduce with age (Kabagambe *et al.,* 1994; Hagisawa *et al.,* 1991). An *in vivo* study in dogs concluded that the reactive hyperaemic response appears to be mediated by the lack of blood oxygen saturation rather than by an increase in blood carbon dioxide concentrations (Crawford *et al.,* 1959). Ross *et al.* (1962) carried out a similar experiment using four dogs and concluded that lack of oxygen during arterial occlusion resulted in a relaxation of smooth muscle surrounding the vessels and the resulting hyperaemic response post removal of the arterial occlusion. In tests where the blood oxygen was maintained at 100% post-occlusion, there was a four-fold increase in blood flow, which returned to baseline within 5 minutes. In tests were blood oxygen levels were maintain at zero post-occlusion, the

hyperaemic response also rose four-fold but did not returned to baseline until blood oxygen levels were increase. To show that the effects of local oxygen saturation on the smooth muscle surrounding blood vessels may regulate blood flow locally, Carrier *et al.* (1964) carried out an *in vitro* study using arterial vessels of various sizes. The experiment showed that low oxygen concentrations around the vessels caused the vessels to dilate and high oxygen concentration around the vessels caused them to vasoconstrict. Since the vessels were not surrounded by soft tissue, metabolites where not responsible for such arterial wall reactions. Thus, this suggests that lack of oxygen appears to be related to increased blood flow to nourish the tissues and is referred as the "oxygen lack theory of vasodilatation". However, the post-ischaemia hyperaemic response may also be mediated by metabolites produced locally by ischaemic tissues and by compression of vessels and extra-vascular tissues (post-compression hyperaemia is described later) (Weiss, 1986; Rodbard, 1971[a]). A study investigated the effects of ischaemic insults to the leg upon skin blood flow in the forearm of 19 healthy subjects using venous occlusion plethysmography. An increase in forearm skin blood flow occurred 30 to 60 seconds following the release of the leg cuff suggesting that local vasodilatating metabolites released by the ischaemic tissues in the leg become blood borne and have a vasodilatating effects on other tissues in the body (Freeburg *et al.,* 1960). Following an extensive literature search no other publications reporting these findings were found. Thus, in chapter 6 the equipment was modified by adding a control probe to detect whether externally mediated pressure induced ischaemia on the plantar aspect of the heel causes a vasodilatating effect on the forefoot of the same foot.

A study by Colin *et al.* (1996) measured the effects of applying external pressure over sacral skin using an indenter and was able to quantify the effects of pressure on simultaneously measuring transcutaneous oxygen tension (Microgas 7640 Kontron Instruments, UK) and skin blood flow (using a laser Doppler fluxmeter, Periflux PF2B, Perimed, Sweden). Following a baseline recording, pressure was applied in steps at two-minute intervals up to 23.4 kPa. A significant decrease in blood flow occurred at 2.7

kPa and at 5.3 kPa a significant decrease in cutaneous oxygen occurred. This suggests that external pressure has the effect of reducing or arresting the nutritive blood flow to the living tissues. Following the disturbance of the nutritive flow, starvation of the cells commences during this ischaemic phase as tissue oxygen saturation decreases. Finally, when the soft tissues are subjected to pressure for a short time period the resultant hyperaemic response is sufficient to compensate for the temporary ischaemia (Livingston, 1992).

3.10.2 Development of ulcers

It only requires a small amount of pressure maintained for a long period of time or a high pressure over a short period of time to deprive the tissues of their nutritive blood supply and develop ischaemic necrosis (Colin *et al.,* 1996; Kabagambe *et al.,* 1994; Livingston, 1992; Ek *et al.,* 1984; Bauman *et al.,* 1963). In addition, a number of other factors contribute to the development of ulceration: the presence of a bony, weight-bearing prominence close beneath the skin; the tissue padding thickness between the bone and skin; lower limb muscle dysfunction; endothelial function; and finally, the skin's integrity (Sigaudo-Roussel *et al.,* 2004; Koitka *et al.,* 2004; Fromy *et al.,* 2002; Abboud *et al.,* 2000; Kabagambe *et al.,* 1994; Livingston, 1992). In animal *in vivo* studies Willms-Kretschmer *et al.* (1969) found fat cells and striated muscle cells to be much more sensitive to ischaemia than skin. In the rat's leg Husain (1953) found striated muscle to be affected by localised pressure before the skin. In addition, because the fat and striated muscle cells were often complicated with post-ischaemic necrosis, the overall resistance of the skin to ischaemia was reduced (Daniel *et al.,* 1981; Willms-Kretschmer *et al.,* 1969; Husain, 1953). Thus, the skin appears to be least affected by pressure ischaemia, with pressure induced ischaemic necrosis commencing in fat and striated muscle first and subsequently progressing towards the skin with increasing pressure and/or duration (Daniel *et al.,* 1981). Another interesting finding is that bacteria seems to colonise sites of pressure and may also be involved in the process of soft tissue breakdown. In animal experiments Husain (1953) injected intravenously a serum broth culture of *streptococci*, followed by the

application of 100 mmHg (13 kPa) of pressure for 2 hours. Three days later when the blood was sterile no bacteria was present in the heart muscle or muscle in the contra-lateral control leg. However, gram-positive cocci were found on the abscess that developed in the pressure area. The experiment was repeated by applying pressure to the tissues twenty-four hours after injecting the bacterial culture with the same results. The experiment was repeated a third time but pressure was applied three days after injection of the bacterial culture when the blood was sterile and no abscesses or bacteria was found at the pressure site (Husain, 1953). This suggests that high-risk patients, especially those taking drugs that may mask signs of infection, may be at more risk of developing ulceration. The fact that capillary permeability is increased following pressure-induced ischaemia may explain a possible route for any circulating bacteria to become localised at the site of pressure.

In bed-bound patients the posterior surface of the heel is a common site of ulceration due to the lack of soft tissue padding over the bony prominence of the calcaneus (Abu-Own *et al.,* 1995). This may occur as a result of the fat and muscle tissue being compressed between the bone and skin, commencing the process of deep pressure ischaemia. Initially damage to the deeper structures would occur and would later spread to the skin. In comparison, a deep fibrolipid pad and thicker skin protects the plantar surface of the heel, making the soft tissues more resilient to the mechanical stresses of human locomotion. Despite this, some high-risk groups may still develop ulceration on the plantar surface of the heel possibly due to atrophy of the fat pad and other tissues, reducing the protective quality of the heel to dissipate pressure causing deep damage that would later affect the skin and cause ulceration.

3.11 Mechanical pressure, skin blood flow and tissue oxygen

A review of the physiological effects of applied pressure was carried out by Cattell (1936). Living cells may be affected by environmental pressure *per se* in a number of ways, however its effects vary between the various types

of tissues. At cellular level, when external pressure is applied to local tissues there is a displacement of the fluid portions within the cells with the effects most severe at the peripheral of the area being compressed where the gradient is the greatest. In living tissues a small increase in pressure resulted in stimulation of the physiological processes (for example, in the case of muscle contracting under pressure the responses are augmented). The increased physiological reactivity may occur as a result of decreased space between the tissue's molecules that would have the effect of increasing the collision rate between reactants and a shift in tissue fluid from higher pressure to lower pressure (Cattell, 1936). In the food industry high pressure processing (applying pressures of 150 to 550 MPa) is used to inactivate harmful bacteria (Fioretto *et al.,* 2005). This system was adapted by Diehl *et al.* (2005) to destroy micro-organisms in *ex vivo* bones for transplantation in orthopaedic surgery and also to assess the effects of high pressure on the cell viability of human osteoblasts, fibroblasts, and tumor cells. At pressures up to 100 MPa a minute effect on human cell vitality was observed. To affect or destroy human cell vitality pressures between 100 and 400 MPa were applied. Thus, to injure or destroy living tissues pressures have to be greater than those found during human working hence, the direct causes of cellular necrosis and ulceration may not be by the directed effect of external pressure *per se* but as a result of pressure-ischaemia starving the cells of nutrition and leading to cellular death and soft tissue breakdown (Colin *et al.,* 1996; Kabagambe *et al.,* 1994; Livingston, 1992; Ek *et al.,* 1984; Bauman *et al.,* 1963; Cattell, 1936).

Mayrovitz *et al.* (1997) investigated the effects of loading and unloading in heel blood perfusion responses in 11 healthy females using the laser Doppler imager and a clear plastic plate to apply mechanical loads on the heel. The study found a reduction in heel skin blood flow from baseline upon application of pressure, however mechanical pressure was not quantified. The central 10 by 10 mm of the plantar aspect of the heel showed the largest perfusion decrement during loading. During off-loading the central part of the heel also showed the greatest hyperaemic response when compared to the rest of the heel. The study concluded that upon loading

skin blood flow rapidly and significantly reduced in all subjects, more significant in the central loading area of the heel. Off-loading resulted in a significant hyperaemic response. A similar study by Mayrovitz *et al.* (1998) reached the same conclusions as reported above. With the test subjects in a supine position Meinders *et al.* (1996) found that plantar foot pressures above 40 kPa in the centre of the heel are sufficient to arrest skin blood flow and after five minutes of applied pressure a hyperaemic response resulted.

During human locomotion Cobb *et al.* (2001) reported that the short intermittent time scales of pressure loading, on the plantar skin over the metatarsal heads, is not sufficient to induce a reactive hyperaemic response. It appears that during the loading phase of the gait cycle the tissues on the sole of the foot are compressed and skin blood flow is decreased or arrested. The swing phase seems to be the recovery time for tissues, as skin blood flow is restored (Cobb *et al.*, 2001). Rodbard (1971)[a] using an *in vitro* model demonstrated that the mechanism of compression of tissue causes post-compression hyperaemia with the resultant response dependant on the strength and compression of tissues. Although the model was used to describe the effects of muscle compression and relaxation on blood flow the model may be applied to other tissues. Rodbard (1971)[a] explained that when muscle contracts it squeezes the tissues raising extra-vascular pressure. If the compression of tissues is sufficient to raise extra-vascular pressure above capillary pressure, the capillaries collapse and blood flow through them is arrested. The high extra-vascular pressure expresses tissue fluid into the capillaries through filtration/bulk flow and into the blood stream. The quantity of filtrated fluid depends on the strength and duration of contraction (that is, the greater the contraction the high the extra-vascular pressure and the more fluid is pushed through the capillaries; the greater the duration the greater the volume of fluid expressed through). With muscular relaxation, the extra-vascular tissue pressure falls and the capillary transmural pressure becomes positive again causing the capillaries to open and restore blood flow. This causes fluid to leave the capillaries via filtration into the extra-vascular area. The increase in blood flow or post-compression hyperaemic response is dependant on the applied tissue

compression/force, the duration of contraction and the number of such contractions (Rodbard, 1971[a]). Since during human locomotion intermittent plantar foot pressure compresses the tissues on the plantar aspect of the foot, this model may be applied to the effects of walking plantar foot pressure on skin blood flow and fluid exchange.

In a study researching the effects of externally applied pressure on skin surface oxygen content Newson *et al.* (1981) applied pressures of up to 18.8 kPa on the sacrum, greater trochanter and lateral aspect of the thigh in eight healthy participants with a mean age of 26 and no past medical history of Raynaud's disease or scleroderma. The study reported that although it is generally assumed that soft tissues over bony prominences can withstand pressures of 35 mmHg (4.7 kPa) without damage, this is also the case for soft tissue over soft sites. Thus, the fact the Decubitus ulcers occur over bony prominences suggests that the higher pressure over these sites, greater than that in adjacent surrounding tissues, causes a deeper and more pronounced circulatory arrest around the peak pressure apex. The result is a greater tissue ischaemia radiating from the peak pressure apex over the bony prominence. Thus, soft tissue damage occurs from the centre of peak pressure where the effects are the greatest, with a decreasing effect on surrounding tissues with lower pressures. Decreasing skin surface oxygen with increasing pressure and duration of the application of pressure was also found. At approximately 15 kPa over the trochanteric area skin oxygen tension was depressed to an anoxaemic state (Newson *et al.*, 1981).

The effects of weight-bearing pressure on plantar skin blood flow in ten patients with diabetes mellitus with severe neuropathic and autonomic neuropathy and eight healthy controls were assessed using the EMED Gait Analysis force plate and fluorescein flowmetry. The study reported that plantar skin blood flow was arrested in all subjects upon weight-bearing pressures above 3 Ncm^{-2} (300 kPa) and the areas where this pressure was exceeded were the hallux, the metatarsal and heel regions. Peak pressures were the highest in previously ulcerated areas in the diabetic patients

(Proano *et al.,* 1992). However, plantar foot ulcers do not occur at random over these three sites but at peak pressure points, especially in persons with diminished pain sensation, suggesting that the higher the pressure the greater the soft tissue ischaemic damage. Schubert *et al.* (1989) found that the effects of pressure on skin blood flow were different between a bony site (the sacrum) and a soft site (the gluteus region). On the bony area skin blood flow decreased significantly with increasing pressure but over the soft area less variation as a function of the applied pressure was demonstrated. In addition, the level of this pathogenic peak pressure varies from individual to individual and is possibly dependant on the state of the microcirculation (for example, endothelial function) and its ability to respond and recovery from ischaemic injury.

Fromy *et al.* (2002) found that skin blood flow responses to applied pressure were diminished in diabetic subjects with and without sensory neuropathy as compared to healthy controls. In addition, Koitka *et al.* (2004) found that diabetic subjects with impaired endothelial function had impaired pressure induced vasodilation response, this "protective" vasodilation response occurs in response to the initial application of low pressure in healthy subjects. Abraham *et al.* (2001) found that following the pressure induced vasodilation response occurring during the application of low pressure; at pressures of 4 and 8 kPa skin blood flow was impaired. In diabetic mice, Sigaudo-Roussel *et al.* (2004) showed that this protective pressure induced vasodilation was completely abolished in 1-week diabetic mice with endothelial dysfunction but no neuropathy suggesting that this mechanism could be involved in the complex pathway of diabetic foot ulceration. However, none of the above studies were carried out in the foot. Koitka *et al.* (2004) reported that the pressure induced vasodilation response in the plantar aspect of the foot only occurs at skin temperatures above 33.9 degrees Celsius.

3.12 Conclusion

Man has an erect posture, thus raising some interesting questions regarding the distribution of pressure due to body weight acting on the foot and

ground and how this affects its soft tissues. The anatomy and physiology of the foot, especially its soft tissues and circulation is complex, with a web of compensatory inter-related functions that maintains homeostasis. During locomotion the skin can tolerate intermittent high pressure for short periods of time without damage. When static standing pressure on the skin of the soles of the feet appears to cause ischaemia, however shifting the weight from one foot to the other restores blood flow and allows recovery of the tissues easing the discomfort of standing. Where nutritive lack of oxygen occurs, a reactive hyperaemia develops to restore nutritional levels to the starved tissues with the extent and duration of blood flow being proportional to the needs of the tissues. The lack of publications with regards to the effects of external pressure on skin blood flow and its relationship to tissue viability, highlights the importance of not just investigating plantar foot pressure but also how it affects skin blood flow and the viability of tissues within the foot.

CHAPTER 4

PRELIMINARY DEVELOPMENT AND VALIDATION OF A SYSTEM TO MEASURE THE EFFECTS OF PLANTAR FOOT PRESSURE ON SKIN BLOOD FLOW

4. CHAPTER 4: Preliminary development and validation of a system to measure the effects of plantar foot pressure on skin blood flow

4.1 Introduction

For many years the foot, a complex structure that has adapted to support man's bipedal gait, has been the subject of rigorous research although restricted by available equipment and technology at the time. Plantar foot pressure, motion analysis, blood flow, physiology and tissue viability are amongst the areas of most interest. Equipment to measure plantar foot pressure has seen an evolution from mechanical to computerised systems with such systems now widely available for both research and clinical practice. The availability of equipment to measure plantar foot pressure has led to hundreds of research publications and has advanced our understanding of plantar foot pressure distribution in relation to healthy feet and the foot in disease. Similarly, advancements in the speed of cameras and computerisation of motion analysis have lead to significant contributions to our knowledge of foot function. With regards to blood flow, interest has existed since antiquity when man was able to relate palpation of pulses with arterial blood flow. Numerous systems have been developed over the years to measure blood flow from the mechanical stethoscope to the more modern computerised systems. In addition, advancements in technology have allowed both clinicians and researches to be more specific and are now able to measure non-invasively circulation within big and small vessels. Our knowledge of the physiology of the structures within the foot is widespread with thousands of publications, however the interaction between physiological aspects of the foot and dynamic and static plantar foot pressure generated during ambulation or standing is still an area with few studies and where more research is needed. Probably because no commercial systems capable of measuring the effects of plantar foot pressure on skin blood flow are currently available. Thus, in order to investigate the effects of plantar foot pressure on the microcirculation of the foot an experimental system must be developed and validated. The aim of this study is to develop and validate an experimental method that will be

used to measure the effects of quantifiable plantar foot pressure on skin blood flow with the subject in a supine or upright position.

4.2 Development and construction of a system to measure the effects of plantar foot pressure on skin blood flow

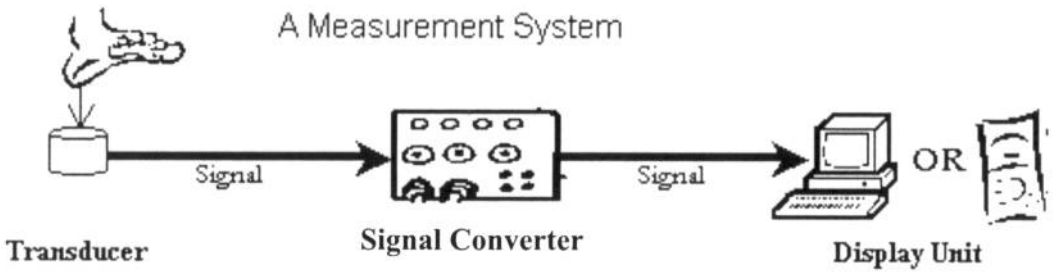


Figure 4-1: General design form of measurement systems.

A number of preliminary investigations were carried out to determine the feasibility of measuring the effects of externally applied pressure on skin blood flow (see Appendix 2). These preliminary investigations showed that the laser Doppler fluxmeter DRT4 (Moor Instruments Ltd., UK) together with a pressure transducer to quantify plantar foot pressure could be adapted to develop a system to measure the effects of plantar foot pressure on skin blood flow.

Following from the preliminary investigations a number of criteria were set for the development of a device to measure the effects of quantifiable plantar foot pressure on the microcirculation of the skin in the centre of the heel (see Table 4-1, page 104). Prior to developing any measurement system it is important to understand the principle behind the operation of such a system. Measurement systems are composed of three basic constituent elements, that is, the transducer, the signal converter and the display unit (see Figure 4-1). The transducer is the sensing element and is responsible for producing a signal related to the quantity being measured, that is, it takes information from the variable being measured (for example, pressure or skin blood flow) and converts it to a different medium (for example, a voltage output) that enables the rest of the system to interpret it and give it a value relative to the quantity being measured. The signal

converter receives the raw signal from the transducer, analyses it and converts it into a form that can be interpreted and given a value by the display unit. The signal converter may be divided into the signal conditioner, the signal processor and the signal transmitter. The signal conditioner is responsible for converting the raw signal received into a physical form suitable for displaying (for example, receiving electrical signals from photo-transducers in the laser Doppler fluxmeter DRT4, interpreting the Doppler shifted raw signals, filtering it and producing a signal suitable for displaying). The signal processor improves the quality of the signal (for example, the low input voltage signal may be amplified into a higher output signal require for normal operation of the display unit). The signal transmitter simply transmits the signal to the display unit and may take many forms (for example, wire cable, optical wire or wireless transmission of the signal). Finally, the display unit receives the output-processed signal from the signal converter and displays it in a form suitable for interpreting (for example, a digital display or computerised or mechanical trace) (Bolton, 2000).

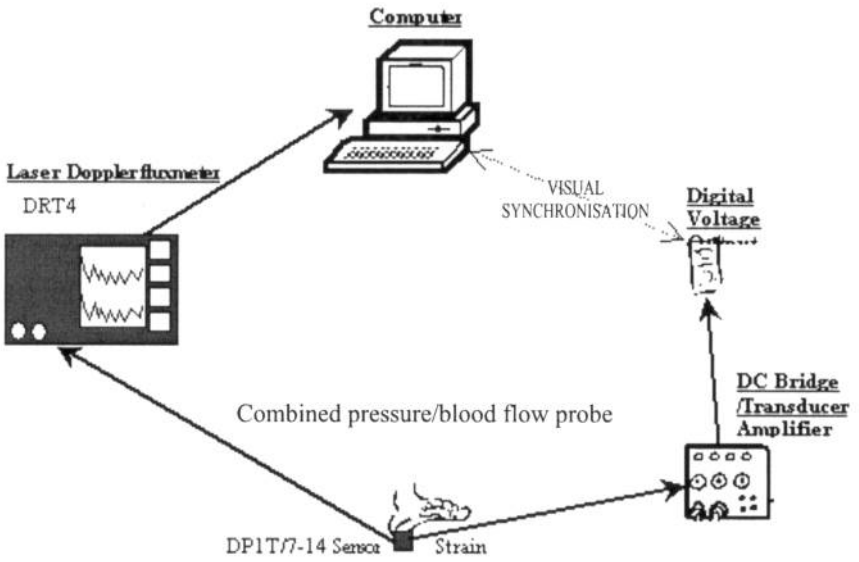


Figure 4-2: Schematic diagram of integration of the laser Doppler fluxmeter with a strain gauge pressure system.

There are currently no systems capable of measuring the effects of quantifiable plantar foot pressure on cutaneous blood flow other than some

experimental models. Following from the preliminary investigations and since two completely separate variables need to be collected (that is, skin blood flow and plantar foot pressure) it is necessary to develop two completely separate systems and then integrate them into one measurement system by synchronising them. Since valid systems suitable for measuring skin blood flow and plantar foot pressure already exist and continuing from the preliminary work, two systems, the laser Doppler fluxmeter (Moor Instruments Ltd, UK) and a strain gauge (Kyowa Electronic Instruments Co., Ltd, Tokyo, Japan) were selected. The aim of the new development was to investigate a method of integrating both systems, calibrate the integrated system and validate the measurement equipment for its new intended application. The rationale for the new development was to integrate the transducers of both systems into one, hence, physiological information for skin blood flow and the environmental information relating to the application or removal of plantar foot pressure could be collected upon the same site simultaneously. The laser Doppler fluxmeter system (fully operational with transducers, signal converter and display unit) has been extensively validated and is extensively used for research and clinical applications, with established calibration methods. Thus, the main development work consisted of integrating the laser Doppler fluxmeter transducer into the in-shoe piston device. However, the pressure system had to be specially developed for the study and involved extensive development work, design of a calibration system and protocol, and a validation procedure. In order to marry the information collected from the integrated transducer, both systems were visually synchronised. Thus, the new integrated system was capable of measuring the effects of plantar foot pressure on skin blood flow in the centre of the heel simultaneously (see Figure 4-2).

- Device must be in-shoe
- The shoe must be able to accommodate for metatarsophalangeal joint deformities, which are characteristic of rheumatoid arthritis.
- Device must be capable of left or right foot recordings, thus allowing for foot randomisation during experiment
- Device must be able to be used both with the subject in a supine position and upright semi-weight bearing position
- Device must be able to record standing blood flow in the plantar aspect of the heel of the foot
- Method of recording skin blood flow must be non-invasive
- Device must be capable of recording the following parameters for the microcirculation of the skin over a period of time:
    - Mean blood cell flux (flow)
    - Number or concentration of moving blood cells
    - Mean speed of blood cells
- Device must be capable of recording skin temperature
- Device must be able to apply quantifiable plantar foot pressure that can be recorded electronically and synchronised with the blood flow readings

Table 4-1 Criteria for the development of the device to measure the effects of quantifiable external pressure on the microcirculation of the skin in the plantar aspect of the foot.

4.2.1 Description of laser Doppler fluxmeter

The DRT4 laser Doppler fluxmeter unit (described in section 2.1.2) uses a solid-state diode to produce a red laser light of 780 nanometres. Two glass optic fibres, within each of the probe cables, connect the DRT4 unit. One fibre optic wire 200 microns (that is, 0.2 millimetres) diameter transmits the laser beam to illuminate the tissues. The other 140 microns (that is, 0.14 millimetres) diameter receives the frequency shifted laser light. A photo-detector within the DRT4 unit receives the laser light and an analogue processor amplifies and processes the signal. The processed signal is then sampled and processed a second time by a digital processor. The digital processor is also responsible for performing all the user interface and display functions. The DRT4 unit can be connected to a personal computer to receive real time data, which can be displayed, stored and analysed.

The DRT4 unit calculates the mean blood cell flux (FLUX), number or concentration of moving blood cells (CONC), the mean speed of the moving blood (SPEED), the detected total of backscatter light (DC) and the temperature at the probe/skin interface (TEMP) although not all probes can record temperature.

4.2.2 Construction of the measurement shoe

The first criterion was that the device must be in-shoe, thus, a medical-surgical shoe (Darco International Inc, 1327 Seventh Avenue, Huntington, WV 25701, USA) was selected for two reasons. First, the toe box is open and can accommodate any foot deformity associated with the metatarsophalangeal joints. Secondly, the shoe may be used for the left or right foot. The shoe was modified to remove the rocker-bottom by adhering a layer of Ethyl Vinyl Acetate (EVA). A hole 2.1 centimetres in diameter was drilled through the centre of the heel to accommodate the piston and a groove 2 centimetres in width by 3.2 centimetres in height was cut out on the lateral aspect of the shoe to allow the cables from the piston to exit via the side of the shoe allowing the up and down action of the piston. The shoe was adhered to a piece of Subortholen, 35 centimetres in length by 14 centimetres in width and 0.5 centimetres thick, with a hinge at the end of the shoe toe box to allow lifting of the heel of the shoe and access to insert the piston and transducers. The hinge was fitted to a wooden board, 29 centimetres in width by 50 centimetres in length and 2 centimetres thick, so that the shoe was in the centre. A hole was drilled through the wooden board in line with the hole in the centre of the heel on the shoe to accommodate the piston. The piston pressure mechanism was then fitted to the underside of the wooden board (see Figure 4-3 and Figure 4-5).

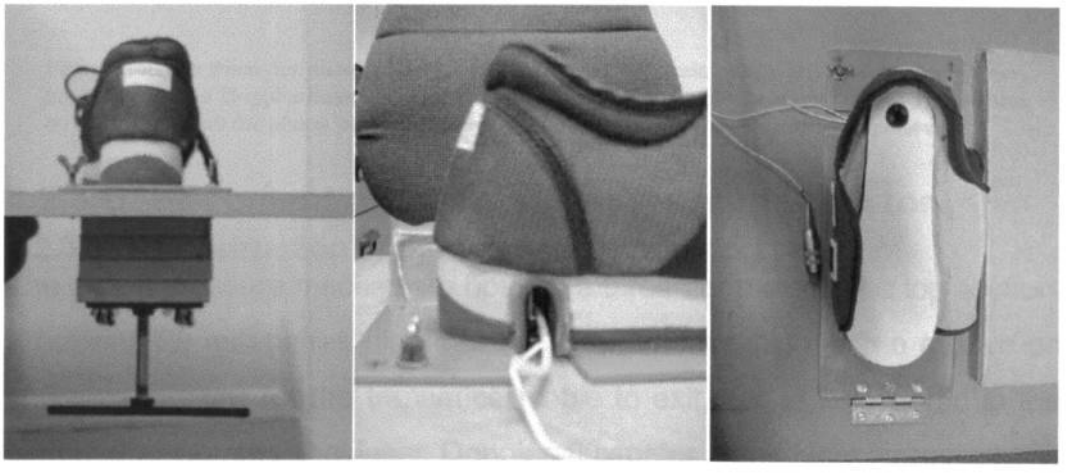


Figure 4-3: Development of the shoe device. The left picture shows the rear view of the device with the shoe on top and the spindle mechanism below. The middle picture shows the side of the shoe with the transducer cables exiting through the groove. The right picture shows the piston in the centre of the heel.

The piston pressure mechanism housed a three-tier piston (described later in Section 4.2.3), which allowed for plantar foot pressure to be applied, removed and quantified by turning the handle using the spindle mechanism (see Figure 4-3 and Figure 4-5). To meet the set criteria that the device must be capable of taking recordings with the subject in a supine position and with the subject sitting with the legs in dependency (that is, in a semi-weight bearing position) a frame and a stand was constructed (described later in section 4.2.4).

In order to measure the amount of pressure applied to the skin, a LM-50KA strain gauge pressure transducer was used. The transducer is 20 (±0.1) millimetres diameter and was placed in the piston below the laser Doppler fluxmeter probe. As pressure was applied the pressure transducer was able to quantify the pressure applied by the piston on the skin (see Figure 4-4 and Figure 4-5).

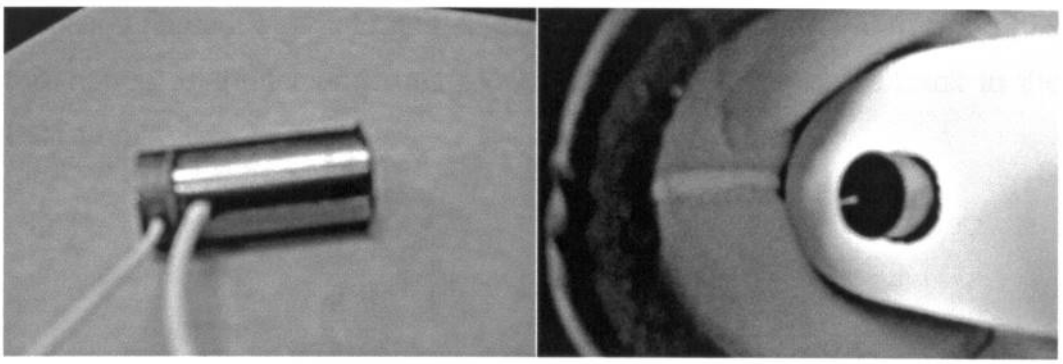


Figure 4-4: The three-tier piston. The left picture shows the strain gauge attached to the top tier, housing the laser Doppler fluxmeter probe. The bottom tier of the piston is not shown. The picture on the right shows the piston protruding through the shoe during the application of pressure.

4.2.3 Development of the three-tier measuring piston

4.2.3.1 Construction of the three-tier measuring piston

The piston pressure mechanism housed a three-tier piston. The top section, a housing 20 millimetres diameter by 38 millimetres high with a groove on one side to allow for the transducer cable to exit, had a hole bored in the centre and housed the laser Doppler fluxmeter transducer 8 millimetres diameter by 34 millimetres high. The middle tier was composed of the strain gauge transducer (see Figure 4-4 and Figure 4-5). A 20 millimetres by 61 millimetres high metal bar composed the bottom tier. The piston was

housed in a cylinder with a nut and plate welded to one end. A spindle mechanism was fitted to one end of the cylinder. By turning the handle on the spindle mechanism clockwise or anti-clockwise, the piston could move up or down through the wooden board and the hole in the centre of the heel in the shoe (see Figure 4-3 and Figure 4-5). Thus plantar foot pressure could be applied or removed to the centre of the heel by turning the spindle mechanism. As pressure was applied the pressure transducer was able to quantify the pressure applied by the piston onto the skin and the laser Doppler fluxmeter transducer simultaneously measured skin blood flow.

4.2.3.2 Description of laser Doppler fluxmeter probe

The modified laser Doppler fluxmeter DP1T/7-14 transducer (www.moor.co.uk, 2004) consists of one central optic fibre 200 microns (0.2 mm) diameter delivering the laser light to the tissues and eight collecting optic fibres 140 microns (0.14 mm) diameter spread evenly forming a circle from the central delivering fibre to a diameter of 2 millimetres. The eight receiving fibres merge into one single receiving optic fibre 140 microns (0.2 millimetres) diameter and runs along the length of the cable back to the DRT4 unit.

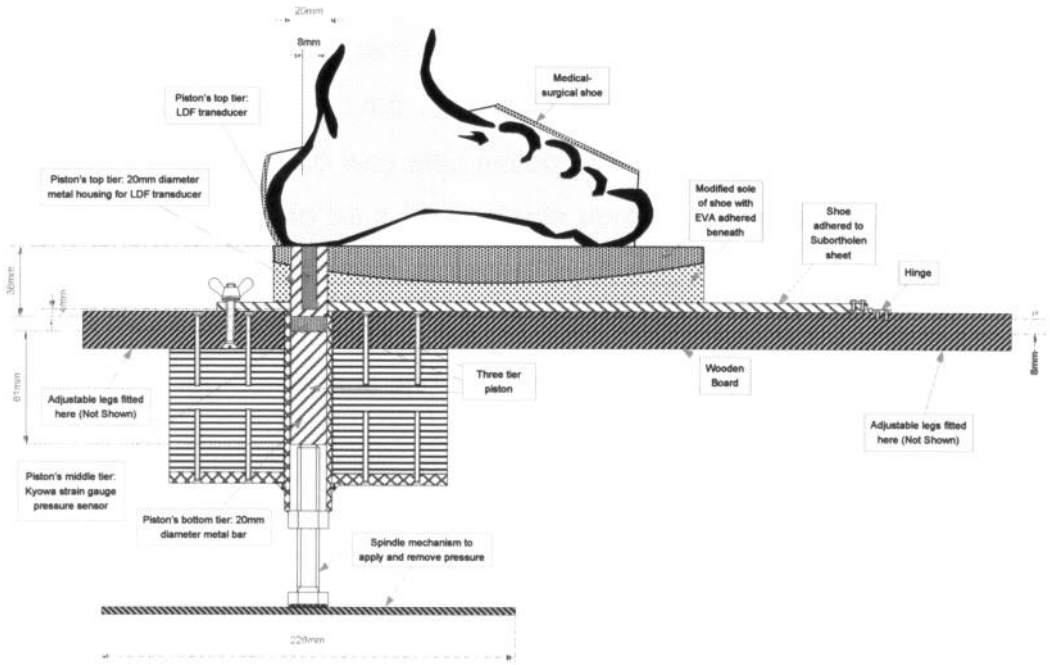


Figure 4-5: Cross-sectional drawing of developed device.

4.2.3.3 Description of Kyowa pressure transducer

The strain gauge pressure transducer was tested to determine its suitability for the study. The transducer weighs 20 grams, provides a rated output of 0.75 to 2 mV/V (1200 to 4000^{-6} strain), rated load of 50 Kgf, and a natural frequency of up to 43900 Hertz. It produces a non-linearity error of ±1% of rated output; hysteresis ±1% of rated output; recommended excitatory voltage 1-5 Volts, alternating current or direct current; safe excitation voltage (maximum) 7 Volts, alternating current or direct current; bridge resistance 350 ±8.75 Ohms; repeatability 1% of rated output; compensated temperature range 0~50°C; safe temperature range 10~60°C; and temperature effect on zero balance ±0.05% of rated output (see Figure 4-6).

Figure 4-6: Kyowa strain gauge used to measure plantar foot pressure.

4.2.4 Development of modular frame and stand for shoe device

The frame was fixed with hinges to the underneath of the wooden board and allowed for the shoe to be positioned at the end of the examination couch almost in a vertical position for supine measurements. By collapsing the frame, the wooden board could be fitted on the stand allowing the shoe to be parallel to the ground for semi-weight bearing measurements (see Figure 4-7). The device was also tested with the subject standing, however the stand appeared to be a bit unstable upon applying full body weight. In addition, since the foot was strapped in, it may have lead to injury if the subject became unbalanced, hence static weight bearing measurement would prove too dangerous and was not considered in this study.

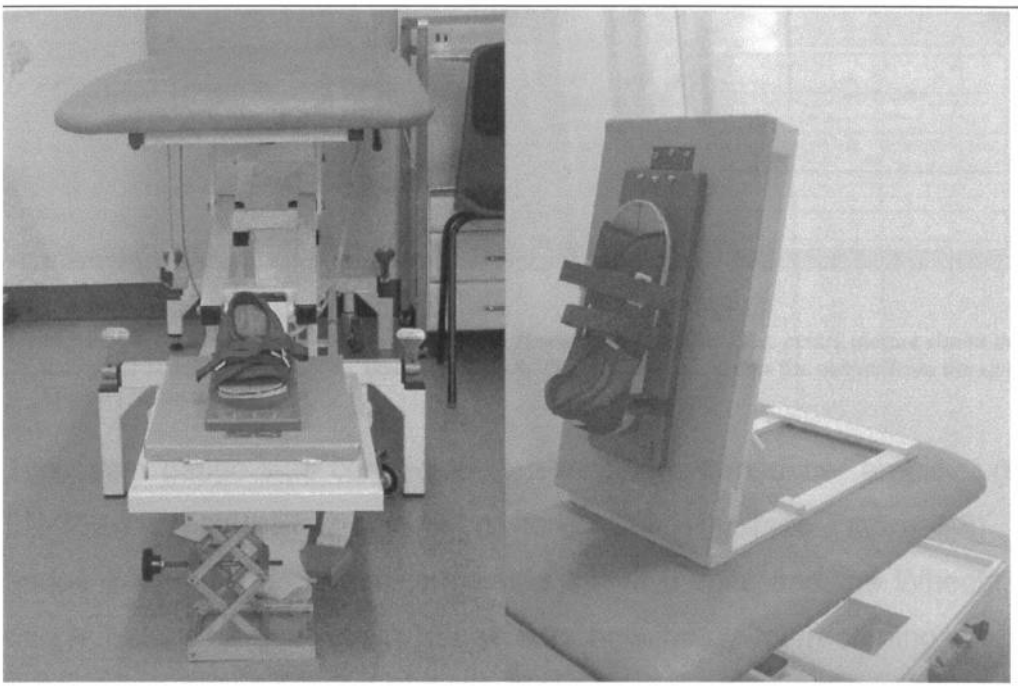


Figure 4-7: The left picture shows the stand that allows for semi-weight bearing measurements and the right picture illustrates the collapsible frame that allows the shoe device to be positioned at the end of a couch for supine measurements.

4.2.5 Assembly of system and data output

The DP1T/7-4 laser Doppler fluxmeter transducer within the three-tier piston was connected to the DRT4 unit. Using an RS232 serial link the data output was sent to a personal computer in real time. The DRTSOFT software installed in the computer allowed for easy analysis of skin blood flow data and markers to be placed during the various data recording events (see Figure 4-8). Using the iontophoresis function within the software, a time dependant period of events could be determined and the software would automatically place markers, which were also signalled with a beep sound. Hence, a baseline period (for example, of 2 minutes) could be determined, followed by a period of external pressure application (for example, of 5 minutes), and then by a hyperaemic response period (for example, of 5 minutes). Since markers were automatically entered the timing of events for all subjects was the same and easy analysis of each separate period was possible (see Figure 4-8).

Figure 4-8: The left picture shows the dc bridge/transducer amplifier. The centre picture shows the computer and the laser Doppler fluxmeter DRT4. The right picture shows the output from the laser Doppler fluxmeter.

The strain gauge was connected to a dc bridge/transducer amplifier (type FE-359-TA, Flyde Electronic Laboratories Ltd., UK) (see Figure 4-8). The bridge supply to the strain gauge was set at 5 volts and the Wheatstone bridge balanced. The strain gauge was now ready for recording and the voltage output is relative to the applied pressure. The voltage output from the strain gauge was connected to a digital display. Both systems were visually synchronised, hence at specific times when markers were placed (at the time of each beep) the voltage output was recorded. The voltage output was then converted to a pressure reading and entered into each marker in the DRTSOFT software for analysis.

A limitation of this system was that both systems were visually synchronised and a time delay human error is generated. Also only one external pressure reading could be entered at each marker and real time continuous external pressure measurement was not possible. A pilot was carried out to determine whether the voltage output analogue signal could be automatically entered and merged with the laser Doppler fluxmeter blood flow output. Using the AN02 unit (Moor Instruments Ltd, UK) it was possible to synchronise both systems in real time and allow continuous recording of skin blood flow and external pressure (see Figure 4-9). The AN02 units allows for 4 analogue inputs and 4 analogue outputs (range 0 to 10 volts) to interface with the DRT4 unit and the computer using BNC connections. This allowed for the analogue voltage output signal from the dc bridge/transducer amplifier unit (received from the strain gauge) to merge with the skin blood flow data and recorded using the DRTSOFT software in real time. Thus skin blood flow and external pressure could be recorded in synchrony and in real

time. However, this set-up was only able to quantify external pressure in a scalar 0.1 volt interval and over a range of 0 to 10 volts it would only produce a resolution of 100 points of data. Over a range of for example 0 to 1000 kPa the system would only be sensitive to ±10 kPa. Hence, the effects of small changes in applied pressure on skin blood flow may not be detected. Thus although this set-up was able to electronically synchronise skin blood flow and external pressure in real time, it was not sensitive enough to quantify external pressure for the purposes of the intended research. Thus, the previous system (see Figure 4-2, page 102) using visual synchronisation and a digital display for voltage output from the strain gauge was adopted.

Figure 4-9: The laser Doppler fluxmeter (Moor Instruments Ltd., UK) AN02 box for analoque signals to integrate with the DRT4 unit.

4.2.6 Method of operation of developed device in supine and semi-weight bearing positions

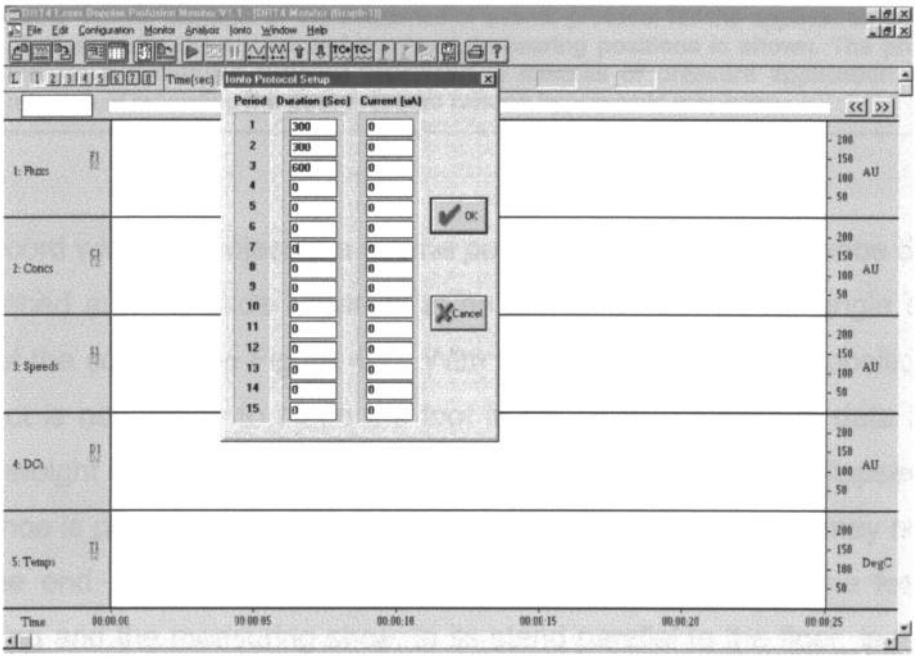


Figure 4-10: A 300 seconds (that is, 5 minutes) period for baseline and pressure applied markers are shown entered in the iontophoresis box. A 600 seconds (that is, 10 minutes) marker is also shown

Prior to data recording, the protocol for the study is determined and the sequence of events is entered into the iontophoresis part of the DRTSOFT software so that markers are automatically placed along the skin blood flow trace at predetermined periods of time (signalled by a beep allowing the researcher to take a recording of the output from the strain gauge) (see Figure 4-10). Using the "Strain Gauge Conversion Calculator" software (developed for the study, see Appendix 3) the values of pressure required to be applied to the skin (in kilo-Pascals) is converted to a voltage reading as determined by the output from the strain gauge equivalent to that amount of applied external pressure (for example, 16 kilo-Pascals is equivalent to 0.143 voltage output). With the study's sequence of events protocol and quantity of external pressure to be applied now established the system is ready for setting up and ready for data collection in the supine or semi-weight bearing positions (see Table 4-2 below).

Session 1 (Supine)	5 minutes baseline measurement (0 Volts of pressure)	5 minutes of pressure application of 16 kPa (i.e. 0.143 Volts)	10 minutes of post-pressure release recording (0 Volts of pressure)
Session 2 (Semi-weight bearing	5 minutes baseline measurement (0 Volts of pressure)	5 minutes of pressure application of 16 kPa (i.e. 0.143 Volts)	10 minutes of post-pressure release recording (0 Volts of pressure)

Table 4-2: An example of the sequence of events protocol for the application of 16 kPa of pressure in the supine and semi-weight bearing positions is shown. The protocols include a 5 minutes baseline recording, 5 minutes of pressure application and 10 minutes of recording the post-pressure release hyperaemic response.

To record with the subject in a supine position the frame on the shoe device is opened and the shoe is set-up at an almost perpendicular angle at the end of the couch (see Figure 4-7). With the subject in a supine position the subject is now ready to place the foot in the shoe. To capture data in the semi-weight bearing position the frame on the shoe device is collapsed and the shoe is placed on the stand parallel to the floor. The subject may now sit on the end of the couch. The couch is then raised so that the feet are hanging and the measuring shoe, in its stand parallel to the floor, is moved beneath. The couch is then lowered slowly whilst carefully placing the subject's foot in the measuring shoe (see Figure 4-7).

With the subject in a supine position on the couch or sitting in the semi-weight bearing position, the randomised left or right foot is inserted into the shoe. To achieve reproducible results the foot is placed in the shoe ensuring the heel is in contact with the heel counter and the midline of the third digit is aligned with the mark on the insole of the shoe prior to holding the foot with the shoe straps. Since the foot is strapped and unable to move it reduces movement artefacts affecting skin blood flow readings.

Prior to commencing the collection of skin blood flow data the digital voltage output is checked to ensure that the pressure acting on the skin is zero. The baseline skin blood flow is then recorded at zero pressure for the period determined by the study's protocol. At the study's predetermined marker for the application of pressure (signalled by a beep) pressure is applied by turning the spindle mechanism beneath the shoe to the predetermined study pressure using the visual digital voltage display. At the next marker (also signalled with a beep) the pressure is removed as quantified by zero volts and the hyperaemic response measured for the predetermined period of time as it returns back to baseline is measured.

4.3 System calibration

Calibration is the process of comparing the measuring system's output against a known standard with the transducer in a defined or controlled environment (Bolton, 2000). Calibration enables the system to be set to reflect the known standard. However, all calibration methods are liable to sources of errors. Sources of error can never be removed completely however, within the study's constrains of available equipment and resources, they were reduced to the minimum possible that were acceptable by study's standard and are reflective of the validation results.

4.3.1 Laser Doppler fluxmeter: Flux calibration using Brownian motion

Calibration of the laser Doppler fluxmeter probes was carried out following the manufacturers' instructions. Ambient lighting was checked since extremely strong ambient lighting may cause errors during the calibration procedure. The background DC levels (that is, detected total back scatter

light) were checked with the laser switched off. Moor Instruments Ltd. (UK) recommends DC levels should not be higher than 1 or 2 units for correct calibration. The DC levels of the calibration room was less than 2 units and this was achieved by switching the room lighting off.

The calibration kit supplied by Moor Instruments Ltd (UK) was placed on a surface free from vibration, as environmental vibration will adversely affect the calibration procedure. The probe was immersed and positioned in the middle of the fluid and held with the probe clamp to avoid oscillations since movement can affect the calibration procedure producing artefact recordings. The laser was turned on. The appropriate channel and probe setting was selected for the probe being calibrated and the calibration program on the DRT4 started. During calibration the room temperature was kept at 21.2°C ±0.7°C.

4.3.2 Laser Doppler fluxmeter: Temperature calibration

Calibration of the temperature probes involves executing the temperature calibration program on the DRT4 and entering the constants supplied by manufacturer for each probe (see Table 4-3 for the constants used for each probe). A study was carried out to check the accuracy of the temperature values by immersing the probes in water at various temperatures and checking the laser Doppler fluxmeter temperature against a mercury thermometer. The laser Doppler fluxmeter temperature values were considered accurate for the intended research studies and were checked on a regular basis for deterioration of accuracy.

Probe 1 Serial number - 00958 Resistance at 25°C - 100.16 KΩ Beta value - 4143	Probe 2 Serial number - 00959 Resistance at 25°C - 100.06 KΩ Beta value – 4143

Table 4-3: The constants supplied by Moor Instruments Ltd for calibrating the temperature probes (type DP1T/7-14).

4.3.3 Development of calibration/testing vice for strain gauge

A calibration vice was constructed (see Figure 4-11) and allowed for the pressure transducer to be calibrated against applied load. The calibration

vice consisted of a wooden base and an "H" shaped frame attached to it. A circular retaining hole (20 millimetres in diameter and 1 millimetres high) held the strain gauge in place and prevented it from moving to prevent artefact readings. Immediately above on the horizontal part of the "H" frame a hole 21 millimetres in diameter, centred with the retaining hole on the base, was drilled. An aluminium bar (of known mass) and/or weights were used to apply quantifiable loads on the strain gauge. With the strain gauge in the retaining hole on the base, the aluminium hollow bar with one solid end was fitted through the hole on the "H" frame with the solid end resting on the strain gauge. Higher quantifiable forces/pressures could be applied to the strain gauge by adding weights to the aluminium bar. Voltage output from the strain gauge was calibrated against applied force/pressure and statistical regression carried out.

Figure 4-11: The in-shoe calibration system. The left picture shows the strain gauge unloaded. The middle picture shows the strain gauge loaded with the aluminum bar. The right picture shows the strain gauge load with weights.

To calibrate the strain gauge within the three-tier piston in the shoe device the "H" frame was removed from the wooden base. The hole on the "H" frame was aligned with the three-tier piston and the frame secured to the shoe device (see Figure 4-11). The strain gauge could now be calibrated in-shoe against applied load (as mention in the previous paragraph) using the aluminium bar and weights and statistical regression carried out.

4.3.4 Strain gauge: calibration

The strain gauge was calibrated in-shoe since this is representative of the pressure-measuring environment for the device. Prior to calibration the dc bridge/transducer amplifier supply to the strain gauge was checked (that is, output must read 5 volts) and the Wheatstone bridge was checked and

balanced if necessary. The calibration process involved testing the pressure system for loading and unloading for a number of repeated measurements. The unloaded strain gauge output was recorded (see Figure 4-11). The calibration aluminium bar of known mass was inserted and the voltage output recorded. Progressively heavier weights were added and the voltage output from the strain gauge recorded at each interval. For each of the unloading measurements, the strain gauge was fully loaded with the aluminium bar and all the weights and a recording of voltage output made. The weights were progressively removed and the voltage output of the strain gauge recorded at each interval. At the end, the aluminium bar was removed and the unloaded strain gauge voltage output recorded.

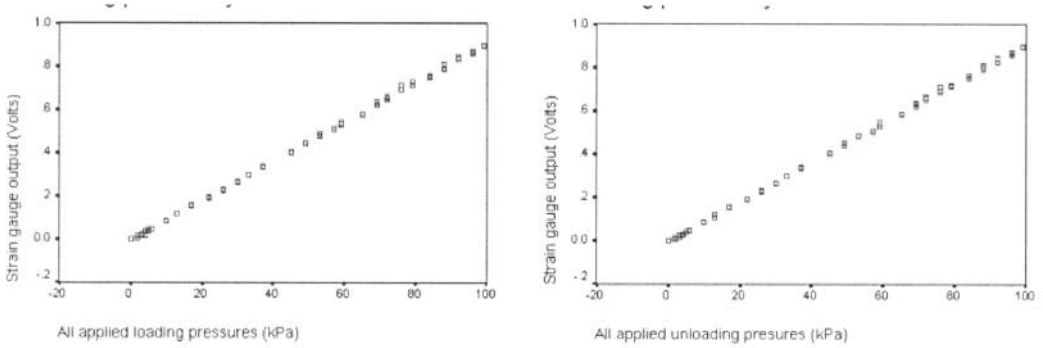


Figure 4-12: The regression for the applied pressures for loading and unloading for the seven measuring sessions.

Linearity refers to the ability of the pressure system to produce test results directly proportional to the true pressure values within a given range. The strain gauge was validated for its loading and unloading linearity over a range of seven repeated tests. The x-axis represents the pressure applied (kPa) to the strain gauge (see Figure 4-12). The y-axis represents the voltage output from the strain gauge. The scatterplots above show a high level of linearity between the strain gauge output and applied loads for both loading and unloading.

	Tests for loading		***Tests for unloading***	
	R^2	Sig.	R^2	Sig.
Method repeatability	0.99	$p<0.01$	0.99	$p<0.01$

Table 4-4: The proportion of variance accounted for by the regression and the significance levels for a regression ANOVA.

The R^2 provides an estimate of the proportion of variance accounted for by the regression. The closer R^2 is to 1 the lesser the proportion of variance to the regression line and the narrower the ellipse of values and the closer to the regression line the points will fall. From the table above it is clear there is a high level of correlation with the applied pressure accounting for 99% of the strain gauge output. A regression ANOVA, which tests the linear relationship between the variables (see Table 4-4), was carried out and shows highly significant results with excellent linearity between the variables as seen in Figure 4-12.

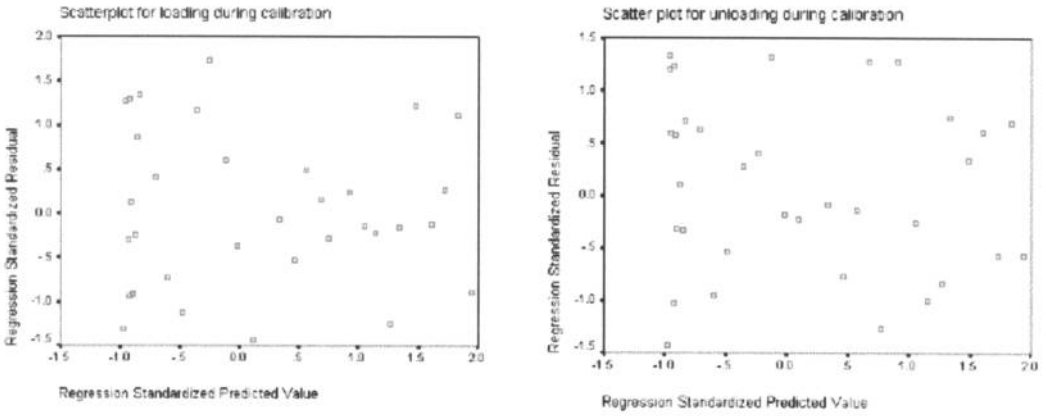


Figure 4-13: Scatter plot of standardised residuals against standardised predicted scores for constant of applied pressure during loading and unloading.

The x-axis (see Figure 4-13) shows the regression standardised predicted value. That is, the mean predicted value subtracted from the predicted value and the difference divided by the standard deviation of the predicted value. Standardised predicted values have a mean of zero and a standard deviation of 1. The y-axis represents the regression-standardised residual. That is, the actual value of the dependant variable minus the value predicted by the regression equation. The scatterplots above show no obvious patterns and confirms the assumptions of linearity and homogeneity of variance for the calibration applied pressure during loading and unloading. If the scatter of points produced a crescent-shaped or funnel-shape the above assumptions of linearity and homogeneity of variance would not have been met (Kinnear *et al.*, 2000).

	Loading	Unloading	Mean
Standard Errors	0.47	0.44	0.46
Maximal residual	0.78	0.59	0.69
Non-linearity Error	0.78%	0.59%	0.69%

Table 4-5: The results show a high degree of accuracy for the calibration of the pressure measuring system. All values are in kPa.

The standard error of the estimate is a good indicator of the accuracy of the regression equation. Assuming Gaussian distribution, the standard error of the estimate represents the mean deviation of the differences between the predicted values on the regression line and the measured values. Ninety-five percent of all systematic errors during calibration are included within two standard errors above and below the regression line. The residual of a point is the measure of the difference between the individual point measured and the predicted point on the regression line along the y-axis. Thus, the maximum residual point shows the maximum difference between the measured value and predicted values (Field, 2002; Kinnear *et al.,* 2000). The maximum residual point is used to calculate the non-linearity error by dividing it by the range and multiplying by 100 (Bolton, 2000). A low standard error of the estimate with a comparatively high maximal residual or non-linearity error suggests that there are a small number of measured values that are different to the predicted regression line. Where the standard error of the estimate and the maximal residuals are similar it suggests that there is a similar trend throughout the measuring range. The lower the standard error of the estimate and the maximum residual or non-linearity error, the higher the degree of accuracy for the calibration system. From Table 4-5, there is little difference between the ±2 standard errors of the estimate and the maximal residuals suggesting that there is a similar trend along the regression line. In addition, 95% of residuals around the regression mean are 0.69% (non-linearity error) of the applied load suggesting a high degree of accuracy for the calibration of the pressure measuring system.

	Tests during loading	Tests during unloading
Measure 1	Y=0.01X+0.01	Y=0.01X+0
Measure 2	Y=0.01X+0.01	Y=0.01X+0.01
Measure 3	Y=0.01X+0.01	Y=0.01X+0.01
Measure 4	Y=0.01X+0.01	Y=0.01X+0.01
Measure 5	Y=0.01X+0.01	Y=0.01X+0.01
Measure 6	Y=0.01X+0.01	Y=0.01X+0.01
Measure 7	Y=0.01X+0.01	Y=0.01X+0.01

Table 4-6: The regression equations obtained from 7 calibration measurements for loading and unloading of the pressure system. Y = strain gauge output (Volts). X = applied pressure (Kilo-Pascals)

Thus, since all the data showed significant correlation with the regression line, the regression equations (see Table 4-6) were produced for calibration measurements with a mean gradient of 0.01, where y is the strain gauge voltage output and x is the applied pressure (kPa). The mean regression equation is Y=0.01X+0.01.

4.3.5 Development of a computerised conversion calculator for mass/voltage to force/pressure

To facilitate the conversion of the voltage output readings from the strain gauge to force or pressure a Microsoft Excel program was developed using the mean regression equation calculated in section 4.3.4 (also see Appendix 3). The program also allowed for the conversion of pressures to be applied to the skin into their equivalent voltage output of the strain gauge. Hence it allowed the researcher to conveniently calculate the loads applied to the skin.

4.4 System validation studies

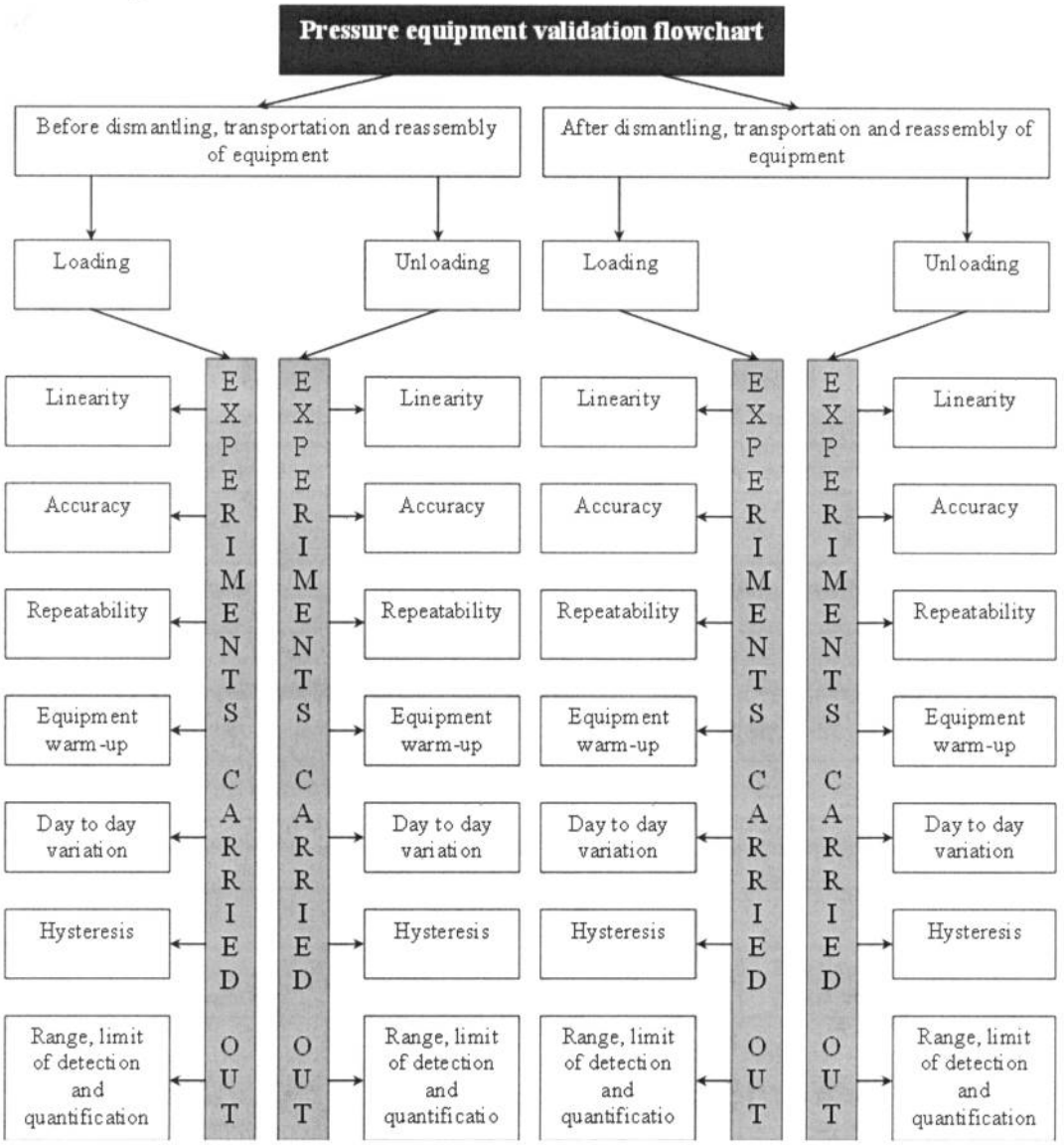


Figure 4-14: Flowchart of the validation experiments carried out for the strain gauge for before and after dismantling, transportation and reassembly of the equipment, for loading and unloading.

To test the robustness of the developed experimental equipment to measure the effects of plantar foot pressure on skin blood flow under conditions similar to those that the equipment will be exposed to in the studies that follow in later chapters, a sequence of validation experiments were designed (see Figure 4-14 and Figure 4-15). The equipment was designed to be portable and hence was tested before and after dismantling, transportation and reassembly. The loading response may vary depending on whether the measured value was obtained by loading or unloading, pressure transducers are particularly prone to this, and hence the loading and unloading response was tested. Within the parameters described

above the equipment was tested for linearity, accuracy, repeatability, effects of equipment warm-up, effects of day-to-day variation, and hysteresis. The tested range, limit of detection and quantification was also determined. The table below (see Table 4-7) provides a quick reference guide to locate the experiments described above for the strain gauge and the laser Doppler fluxmeter.

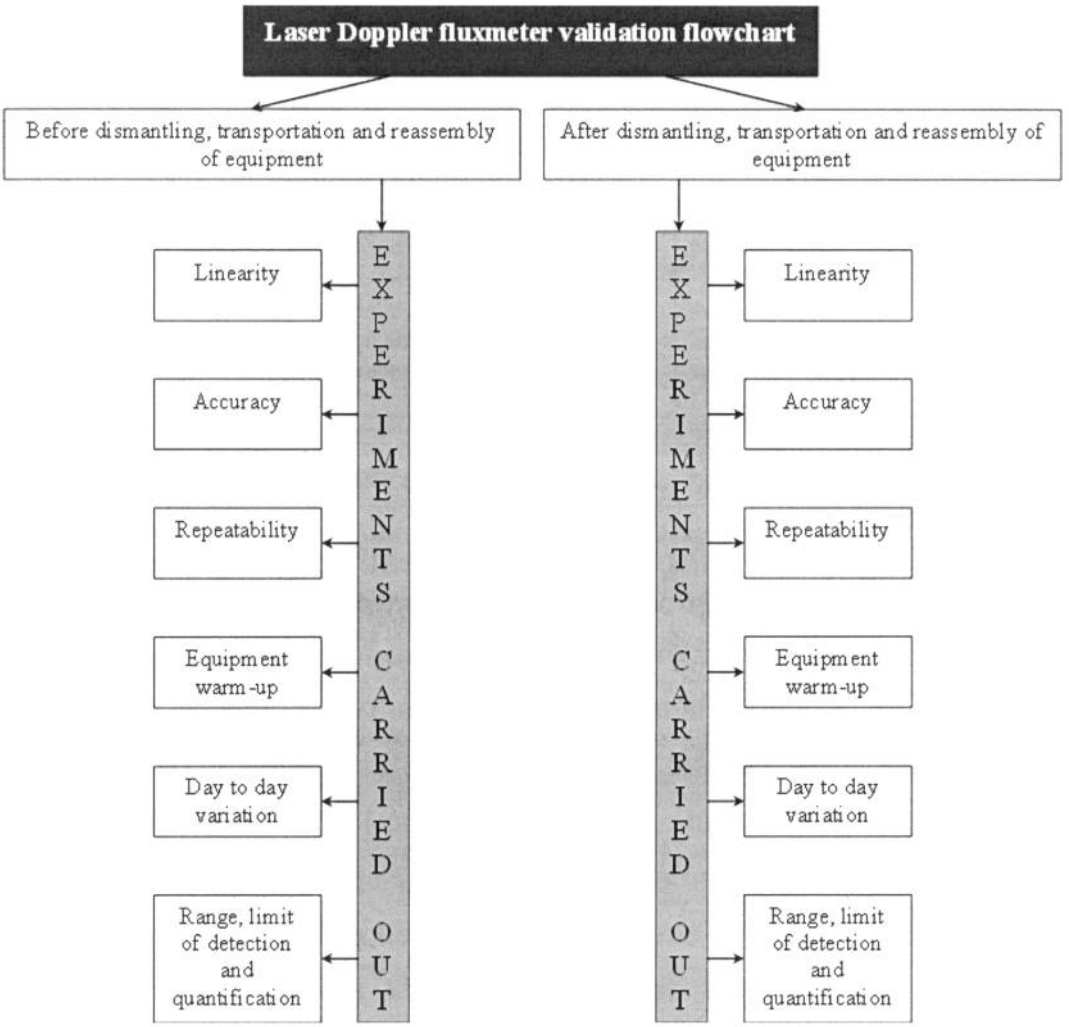


Figure 4-15: Flowchart of the validation experiments carried out for the laser Doppler fluxmeter for before and after dismantling, transportation and reassembly of the equipment.

Experiments	Strain gauge	Laser Doppler fluxmeter
Linearity	4.3.4 Strain gauge: calibration (Page 115)	4.4.2.1 Study 7: Linearity of laser Doppler fluxmeter measurements (Page 140)
Accuracy	4.4.1.1 Study 1: Accuracy of pressure measurements (Page 123)	4.4.2.2 Study 8: Accuracy of laser Doppler fluxmeter measurements (Page 142)
Repeatability	4.4.1.2 Study 2: Repeatability of pressure measurements (Page 125)	4.4.2.3 Study 9 and 10: Repeatability of laser Doppler fluxmeter measurements (Page 148)
Equipment warm-up	4.4.1.3 Study 3: Reproducibility of pressure measurements during equipment warm-up (Page 129)	4.4.2.4 Study 11: Reproducibility of measurements during equipment warm-up (Page 156)
Day to day variation	4.4.1.4 Study 4: Reproducibility of daily pressure measurements (Page 135)	4.4.2.5 Study 12: Reproducibility of daily measurements (Page 161)
Hysteresis	4.4.1.5 Study 5: Hysteresis effects on pressure measurements (Page 139)	Not applicable
Range, limit of detection and quantification	4.4.1.6 Study 6: Range, limit of detection and limit of quantification of pressure measurements (Page 139)	4.4.2.6 Study 13: Range, limit of detection and limit of quantification of laser Doppler fluxmeter (Page 166)

Table 4-7: Quick reference guide to locate the validation experiments for the strain gauge and the laser Doppler fluxmeter.

4.4.1 Validation studies of the strain gauge pressure measurement system

The correct application of quantifiable pressure is a crucial component of the device to measure the effects of external pressure on the skin's microcirculation since incorrect pressures will lead to wrong assumptions. The aim of the pressure transducer validation studies was to evaluate the validity of the strain gauge to quantify the external pressure applied to the skin on the centre of the heel using the in-shoe device. For all the strain gauge validation studies the recordings were taken with the pressure transducer attached to the three-tier piston within the shoe device. Quantified pressure was applied by using the calibration vice attached to the shoe device. Applied mass was converted to applied pressure using the conversion calculator. Similarly, voltage output from the strain gauge was converted to measured pressure (see Appendix 3). Hence, applied pressure could be compared against measured pressure. Prior to each study the system was checked to ensure that an excitation voltage of 5 volts was applied to the strain gauge and the Wheatstone bridge checked and re-balanced if necessary. All studies involved testing the equipment for loading

and unloading, before and after dismantling, transporting and re-assembly. The pressure system was investigated for accuracy, repeatability, reproducibility during equipment warm-up and over a five-day period, and hysteresis.

Dismantling, transporting and reassembling the equipment tested the ruggedness of the pressure system and were measured by repeating all the validation studies described in the above paragraph.

4.4.1.1 Study 1: Accuracy of pressure measurements

The system was set-up as described in section 4.2.5. A Bland/Altman plot was constructed to investigate how much the pressure system's output differed from the known values of applied pressure for both before and after dismantling, transportation and reassembly and for assessing the overall accuracy (see Figure 4-16, page 124). The x-axis represents the mean of the applied and measured pressures. The y-axis shows the difference between the applied and measured pressures. From the Bland/Altman plot the limits of agreement were calculated. The limits of agreement refer to the mean difference plus or minus 1.96 of the standard deviation of the differences that apply to the particular study as a whole. The overall limits of agreement for the tested system were 1.38 kPa above or -1.21 kPa below the known applied pressure (that is, the standard) for the tested range of 0 to 100 kPa (see Figure 4-16). Thus, the equipment is suitable for the intended application since the range of the limit of agreement is acceptable for the proposed use of the measuring system. However, since the limits of agreement refer only to the study data the coefficient of repeatability was also calculated. For repeated measures the coefficient of repeatability refers to the accuracy of the system because the repeatability of the two methods of measurement limit the amount of agreement that is possible. In this case the accuracy of the pressure system against known values of applied pressure was investigated with 95% of differences being less than 1.96 standard deviation thus, an accuracy of plus or minus could be calculated and for the range tested of 0 to 100 kPa the percentage of full-scale deflection could be worked out. The percentage of full-scale deflection is the coefficient of repeatability divided by the tested range multiplied by 100.

	Before transportation			After transportation			Mean of all
	Loading	Unloading	Mean	Loading	Unloading	Mean	
Repeatability	1.30	1.07	1.19	1.13	1.01	1.07	1.13
Warm-up	1.19	1.26	1.23	1.33	1.44	1.39	1.31
Daily variation	1.32	1.31	1.32	1.62	1.37	1.50	1.41
TOTAL	1.27	1.21	1.25	1.36	1.27	1.32	1.29

Table 4-8: All the values shown in the table are in kPa. The coefficients of repeatability for accuracy experiments between applied and measured pressure are shown. The overall coefficient of repeatability for the pressure measuring system is 1.29 kPa.

From Table 4-8 the variation between any of the coefficient of repeatability is small with the mean coefficient of variation for all the investigations being 1.29 kPa with a range of 1.01 to 1.62 kPa. The greatest variation occurred from day to day measurements with a higher mean recorded after transportation and reassembly. The lowest variation also occurred after dismantling, transportation and reassembly of the pressure equipment during the repeatability experiments. Overall, relocating the equipment has minimal effect on the accuracy of recordings and the pressure system is sufficiently accurate for the proposed future studies.

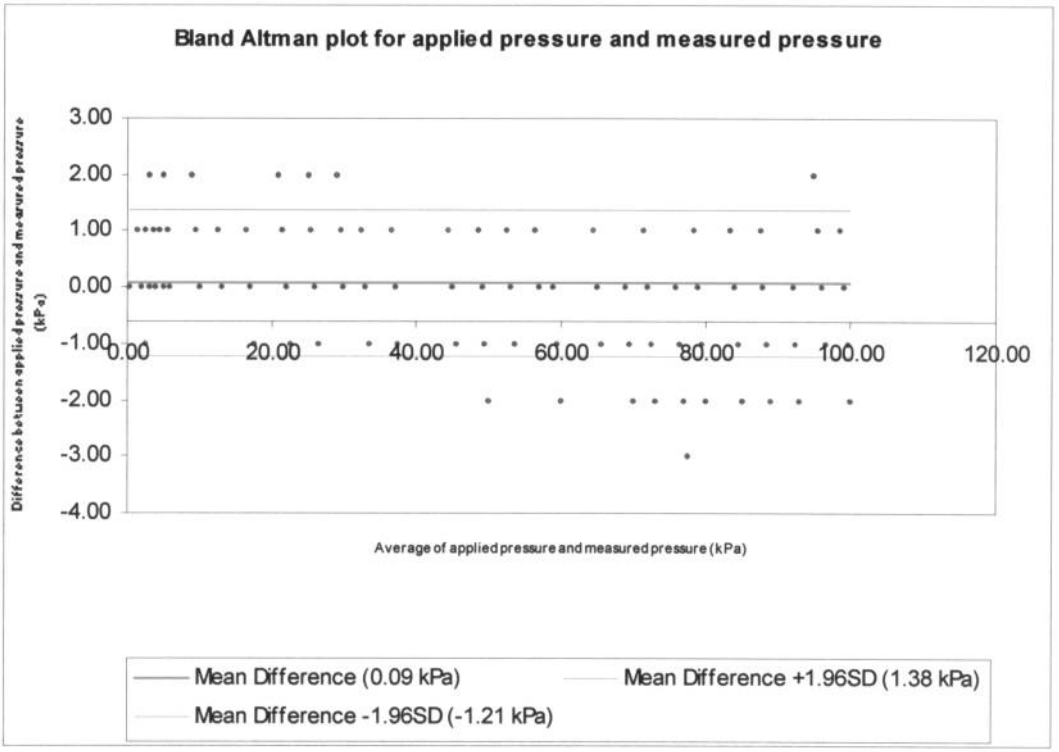


Figure 4-16: The Bland/Altman plot shows the mean and 95% limits of agreement for all the data collected for the validation studies (n=2496).

At a 95% confidence interval the coefficient of repeatability (accuracy) of the pressure system was ±1.29 kPa or ±1.29% of full-scale deflection for the range 0 to 100 kPa. For the study investigating the postural venoarteriolar response the specific pressures applied will be 20, 40 and 80 kPa respectively. The current limits of agreement and level of accuracy of the pressure system suggests that it is suitable to detect any differences between the three applied loads for the study outlined. In addition, using a Pearson correlation the correlation coefficient (Kinnear *et al.*, 2000) for the Bland/Altman plots was calculated to determine the strength of association between the two variables measured. For Figure 4-16 there is a fair correlation between the difference between the applied pressure and measured pressure and the average of the two variables ($r = -0.472$; $P<0.01$). This fair correlation may be accounted by the fact that the applied pressure was applied at selected increasing intervals during the bench testing of the strain gauge transducer.

4.4.1.2 Study 2: Repeatability of pressure measurements

Repeated measurements result in a random error due to factors beyond the control of the experiment that will produce a spread of values. The repeatability study allowed for test re-test of the pressure measurement system under the same conditions to be calculated. Seven repeated measurement sessions were carried out for the range 0 to 100 kPa for both loading and unloading before transportation. The equipment was then dismantled, taken to a new location, re-assembled and a second set of seven repeated measurements was taken for both loading and unloading.

The postural venoarteriolar response study would involve the maximum number of repeated data gathering sessions for both loading and unloading (that is, three supine measurements and three semi-weight bearing). In addition, since the equipment had to be moved during the week it was important to test the ruggedness of the system's repeatability. Hence, for the repeatability validation study seven repeated measures (one more than that required) were taken before and after moving for both loading and unloading to test the repeatability of the pressure system for within subject.

	Coefficient of Variation
BEFORE TRANSPORTATION	
Loading	1.32%
Unloading	1.50%
AFTER TRANSPORTATION	
Loading	1.57%
Unloading	1.47%

Table 4-9: The effects of test re-test on typical errors produced by the pressure system for loading and unloading, before and after transportation are shown. The values for are in kPa.

The coefficient of variation was used to determine the typical error or standard error of measurement for the repeated measures (Batterham *et al.*, 2000). Table 4-9 shows the typical errors arising from repeated measures. The typical errors for loading and unloading for both before and after transportation are low indicating a good level of repeatability for measuring plantar foot pressure.

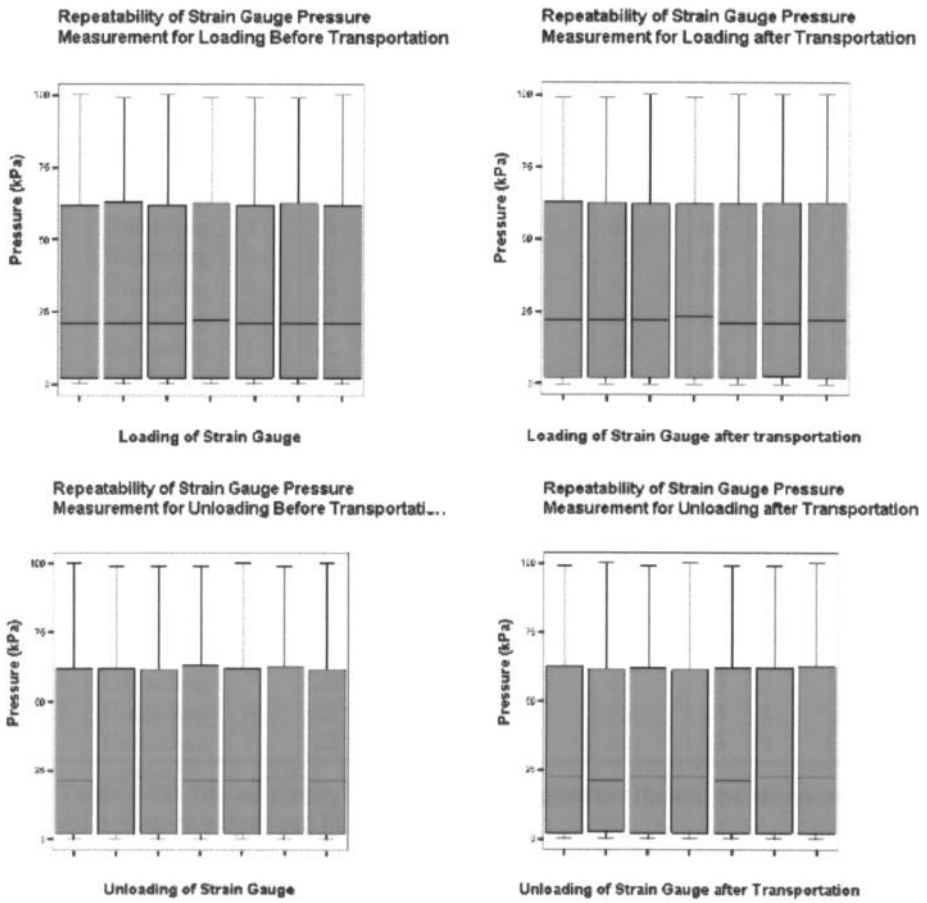


Figure 4-17: The repeatability of seven measurement sessions carried out on the same day.

Figure 4-17 illustrates the measurement of test re-test (that is, repeatability) for loading and unloading before and after transportation, using the strain gauge, that was carried out over four sets of seven measurement sessions on the same day. The x-axis represents the 7 test re-test sessions. The y-axis represents the measured pressure in kilo-Pascals. The red boxplots represent the upper and lower quartiles with the median represented by a black line. The whiskers represent the largest and lowest values. There are no outliers. Table 4-10 (below) shows the median, minimum and maximum values, the 5th, 25th, 75th and 95th percentiles, the interquartile range and the 90% range for the repeatability data presented.

Before or after transport	Loading or unloading	Measuring session	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentiles 5th	Percentiles 95th	90% Range
Before	Loading	1	21	0	100	2	64	62	1	96	95
Before	Loading	2	21	0	99	2	65	63	1	95	94
Before	Loading	3	21	0	100	2	65	63	1	96	95
Before	Loading	4	22	0	99	2	65	63	1	97	96
Before	Loading	5	21	0	99	2	64	62	1	95	94
Before	Loading	6	21	0	99	2	65	63	1	96	95
Before	Loading	7	21	0	100	2	65	63	1	96	95
Before	Loading	1	21	0	100	2	65	63	1	96	95
Before	Unloading	2	22	0	99	2	64	62	1	95	94
Before	Unloading	3	22	0	99	2	64	62	1	97	96
Before	Unloading	4	21	0	99	2	65	63	1	95	94
Before	Unloading	5	21	0	100	2	65	63	1	96	95
Before	Unloading	6	22	0	99	2	65	63	1	96	95
Before	Unloading	7	21	0	100	2	64	62	1	96	95
Before	Unloading	1	22	0	99	2	65	63	1	97	96
After	Loading	2	22	0	99	2	64	62	2	95	93
After	Loading	3	22	0	100	2	64	62	2	96	94
After	Loading	4	23	0	99	2	65	63	1	96	95
After	Loading	5	21	0	100	2	64	62	1	97	96
After	Loading	6	21	0	100	2	65	63	1	96	95
After	Loading	7	21	0	100	2	64	62	1	96	95
After	Loading	1	21	0	100	2	64	63	1	96	95
After	Loading	2	22	0	99	2	64	62	1	96	95
After	Unloading	3	22	0	100	2	64	62	1	97	96
After	Unloading	4	21	0	99	2	65	63	1	96	95
After	Unloading	5	22	0	99	2	65	63	1	96	95
After	Unloading	6	22	0	99	2	65	63	1	95	94
After	Unloading	7	22	0	100	2	65	63	1	97	96

Table 4-10: The summary of results for all repeated measures taken on the same day. All pressure values are in kilo-Pascals.

The four boxplots and above table for all measuring sessions show consistent repeatable results with little difference between the tests (see Figure 4-17 and Table 4-10). The medians show closeness of agreement with a minimum value of 21 kPa and maximum value of 23 kPa. The 90% range, interquartile range, percentiles presented and minimum and maximum values for all recording also show closeness of agreement and consistency. The boxplots are all skewed towards the lower end of the range reflecting that more values were recorded in the lower end of the scale and does not show a true skew as would be seen in a population study.

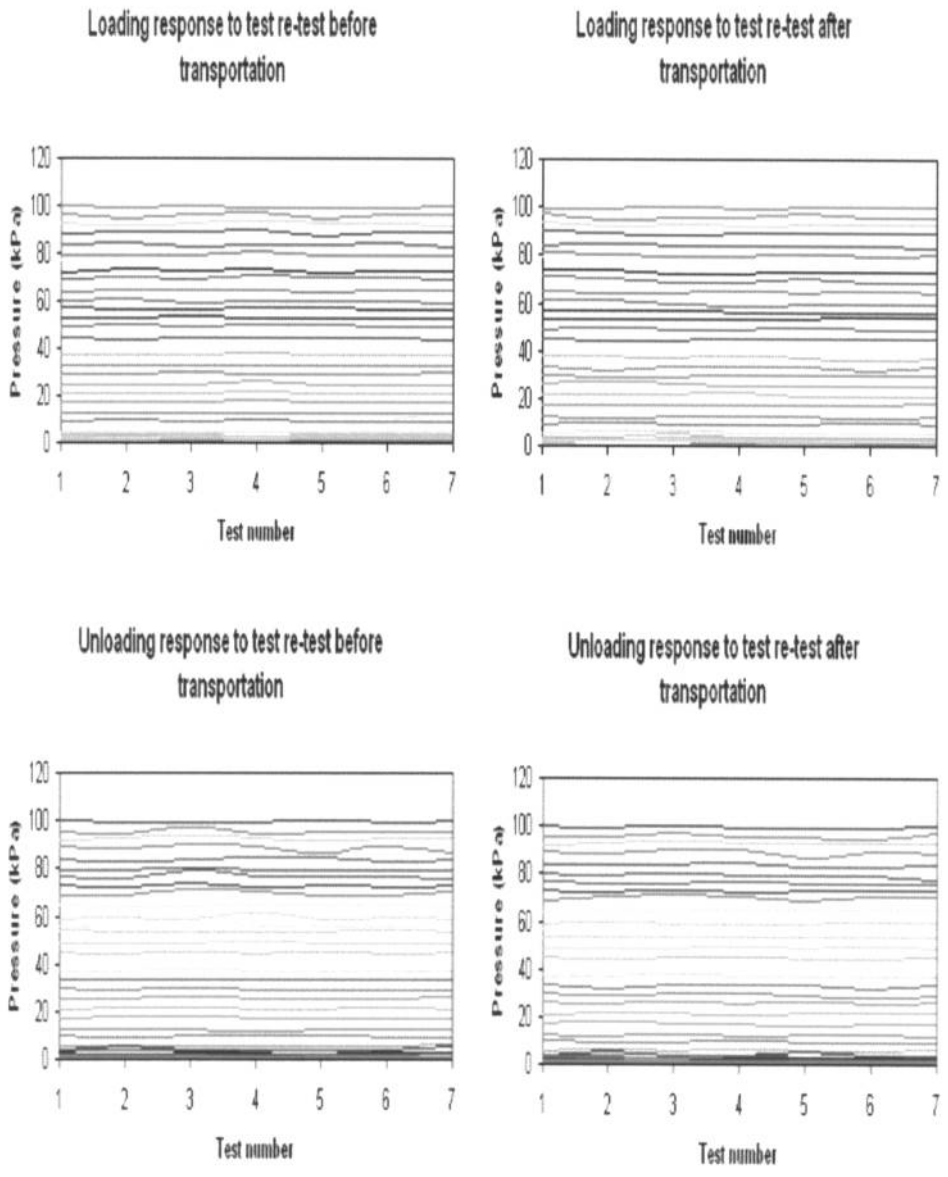


Figure 4-18: Line graphs representative of 7 test re-test measurement sessions. Test number 1 to 7 represents each measurement data recording session.

Line graphs were constructed to investigate individual test re-test pressure values (see Figure 4-18). The x-axis represents the repeatability test with test numbers 1 to 7 representing each of the tests carried out. The y-axis represents the measured pressure in kilo-Pascals. The top two graphs represents the loading response and the bottom two the unloading response. The left two graphs illustrate repeatability of the pressure measuring system before and the right two after relocating. Overall, the line graphs indicate consistency in repeatability of pressure measurements.

Further analysis was carried out by investigating the within measurements median, minimum and maximum values and the 5^{th}, 25^{th}, 75^{th} and 95^{th} percentiles and the interquartile and 90% range for loading and unloading before and after transferring the equipment to a new location (see Appendix 4). The results show a high degree of repeatability for the tested range of 0 to 100 kPa.

In summary, the pressure measuring system shows consistency and repeatability of test re-test under the same controlled operating conditions. Similarly, loading and unloading response shows high levels of repeatability. Disassembly, transportation and reassembly of the pressure system appear to have a negligible effect on repeatability.

4.4.1.3 Study 3: Reproducibility of pressure measurements during equipment warm-up

Since equipment warm-up could have an influence on the strain gauge's output a study was carried out with the aim of determining the effects of equipment warm-up on repeated measurements. The pressure system was set-up in-shoe as described in section 4.2.5. The power was then switched on and baseline measurements taken for incremented loading. Three further repeated loading sessions were taken, for loading of the strain gauge, at intervals of one hour. The power to the strain gauge was left on for the duration of the trial.

The pressure system was then switched off and allowed to cool down for a period of two hours. The strain gauge was then fully loaded, the power

switched on and baseline measurements taken for incremental unloading. As previously, three further measurements were taken at one hour intervals.

Finally the system was dismantled, transported and re-assembled and re-tested for repeated measures for loading and unloading. Results were compared for the effects of equipment warm-up on reproducibility of results before and after transportation and overall.

	Coefficient of Variation
BEFORE TRANSPORTATION	
Loading	1.03%
Unloading	0.97%
AFTER TRANSPORTATION	
Loading	1.12%
Unloading	1.14%

Table 4-11: The effects of equipment warm-up on typical errors produced by the pressure system for loading and unloading, before and after dismantling, transportation and reassembly is shown. The values are in kPa.

The effects of increasing temperature on typical errors during measurements taken using the strain gauge are shown in Table 4-11 above. After relocating the equipment there is a small increase in the typical error for both loading and unloading. However, this small increase is insignificant since they are very small and will have no impact on the studies in Chapter 5 and 7 were varying pressures of 20 kPa, 40 kPa, 80 kPa, 90 kPa and 100 kPa were measured. Thus, equipment warm-up over three hours has a negligible effect on reproducibility of pressure measurements.

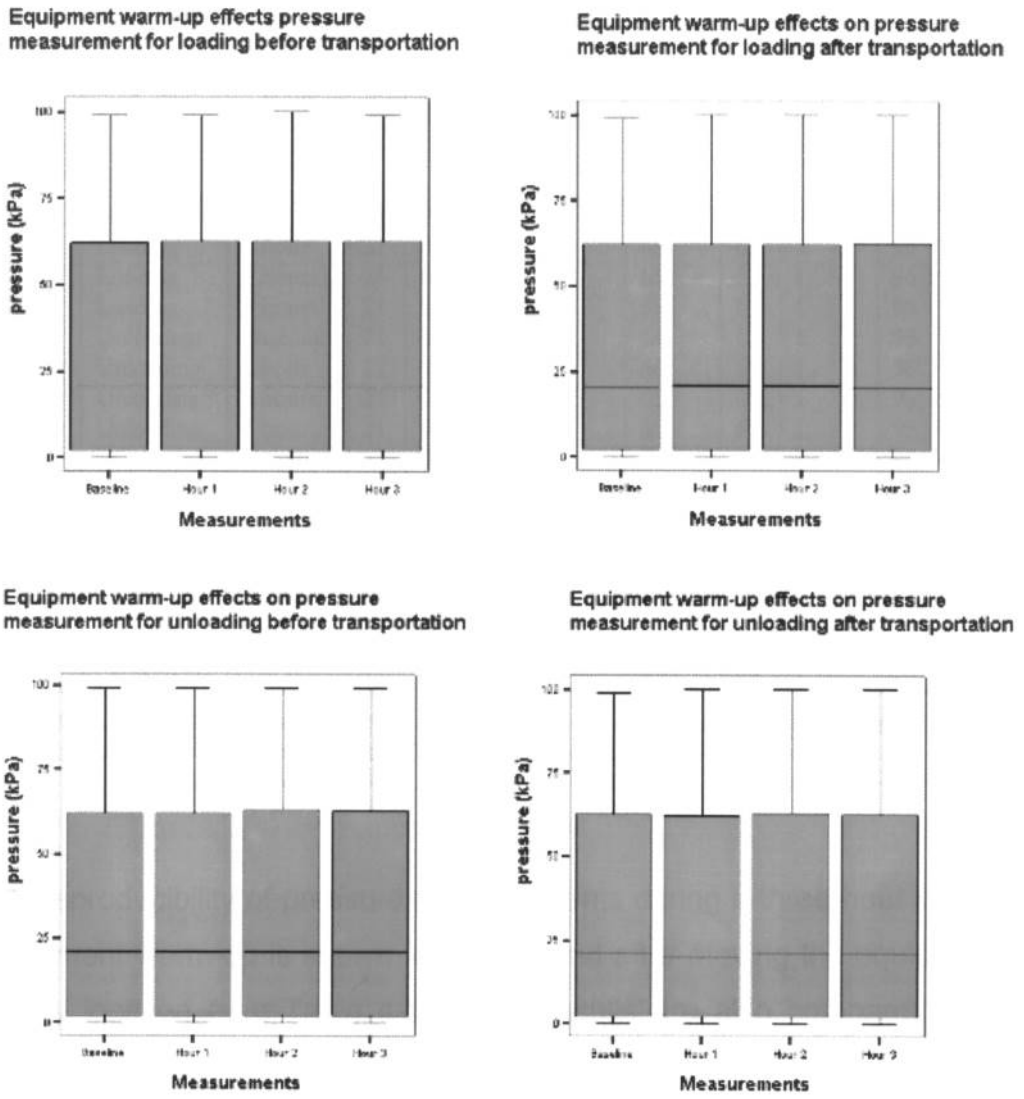


Figure 4-19: Boxplots for loading and unloading, before and after transportation are shown for the effects of equipment warm-up on pressure measurements with the strain gauge.

Before or after transport	Loading or unloading	Measuring session	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentiles 5th	Percentiles 95th	90% range
Before	Loading	Baseline	21	0	99	2	64	62	1	95	94
Before	Loading	1 hour	21	0	99	2	65	63	1	96	95
Before	Loading	2 hours	21	0	100	2	65	63	1	96	95
Before	Loading	3 hours	21	0	99	2	65	63	1	96	95
Before	Unloading	Baseline	21	0	99	2	64	62	1	95	94
Before	Unloading	1 hour	21	0	99	2	64	62	1	96	95
Before	Unloading	2 hours	21	0	99	2	65	63	1	96	95
Before	Unloading	3 hours	21	0	99	2	65	63	1	95	94
After	Loading	Baseline	20	0	99	2	64	62	1	95	94
After	Loading	1 hour	21	0	100	2	64	62	1	96	95
After	Loading	2 hours	21	0	100	2	65	63	1	96	95
After	Loading	3 hours	20	0	100	2	65	63	1	96	95
After	Unloading	Baseline	21	0	99	2	65	63	1	95	94
After	Unloading	1 hour	20	0	100	2	64	62	1	96	95
After	Unloading	2 hours	21	0	100	2	65	63	1	96	95
After	Unloading	3 hours	21	0	100	2	65	63	1	96	95

Table 4-12: The summary of results for all equipment warm-up measures taken on the same day over a 3-hour period. All pressure values are in kilo-Pascals.

The reproducibility of pressure measurements during a three-hour period of equipment warm-up is shown for before and after moving the equipment to a new location (see Table 4-12). The results are also compared for the effects of loading and unloading on reproducibility of pressure measurements. Figure 4-19 illustrates boxplots for pressure measurements at baseline, after one hour, after two hours and finally, at the end of a three-hour period, where the equipment was kept switched on for the duration of the experiments. The boxplots on the left illustrates results before disassembly, transportation and re-assembly and the boxplots on the right after. The x-axis represents the 4 repeated measurements recorded during the equipment warm-up. The y-axis represents the measured pressure. Table 4-12 shows results for the median, minimum and maximum value, 5^{th}, 25^{th}, 75^{th}, and 75^{th} percentiles, the interquartile and 90% ranges.

The boxplots for the effects of equipment warm-up on the reproducibility of pressure measurements indicate close median values. These median values were 21 kPa for all loading and unloading values before relocating the equipment, and 20 or 21 kPa for all values after suggesting that the

disassembly, transportation and re-assembly of the pressure system has a small effect on reproducibility of pressure measurements during equipment warm-up (see Table 4-12). The interquartile ranges were 62 or 63 kPa with a pattern of an increase in 1 kPa after the equipment had warmed-up over a 1 to 2 hours period observed in the experiments before transportation and only in the loading response after transportation. This pattern was also observed in the 90% range after one hour of equipment warm-up in the loading response before transferring the equipment and unloading and loading after transportation. Thus overall, there is a trend of increasing ranges by 1 kPa during equipment warm-up suggesting that temperature appears to affect pressure measurements. This trend is clear in all the loading responses but not in the unloading "before" response suggesting that the strain gauge may be affected by hysteresis. Overall, the boxplots, median values and ranges indicate that the reproducibility of pressure measurements during equipment warm-up is consistent, with test re-test reproducibility highly reproducible after equipment has warmed up for one hour.

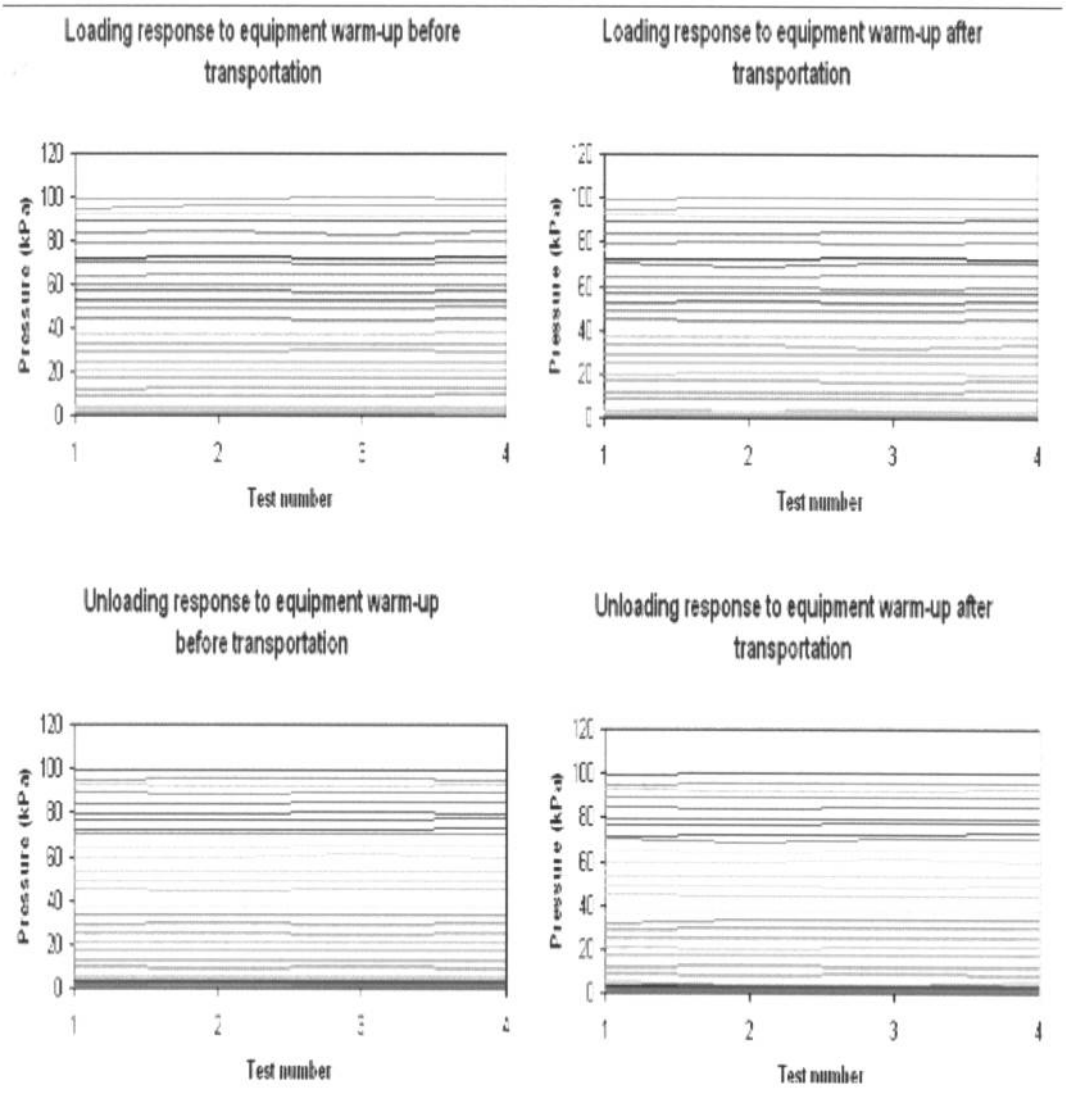


Figure 4-20: Line graphs representative of 4 repeated sessions for equipment warm-up. Test number 1 represents the baseline recording when equipment was switched on. Test number 2 to 4 represents measurements at 1, 2 and 3 hour intervals.

To investigate individual repeated measured pressure values line graphs were constructed (see Figure 4-20). The x-axis represents the test re-test sessions with test number 1 being the baseline pressure measurement when the equipment was switched on followed by measurements at 1, 2 and 3 hours intervals. The y-axis represents the measured pressure. The top two graphs represents the loading response and the bottom two the unloading response. The left two graphs illustrate the effects of equipment warm-up before and the right two after reassembling the strain gauge. Overall, the line graphs indicate consistency in the within reproducibility of pressure measurements.

Further analysis was carried out by investigating the within measurements for median, minimum and maximum values and the 5th, 25th, 75th and 95th percentiles and the interquartile and 90% range for loading and unloading before and after transportating the pressure system (see Appendix 4). The results show a high degree of repeatability for the tested range.

An insignificant rise in pressure values following an increase in the temperature of the strain gauge was noticed but such small variation is acceptable for the planned studies investigating the postural venoarteriolar response and also in rhueumatoid arthritis. However, since the strain gauge has been shown to be affected by equipment warm-up (although minimally) and stabilises after one hour it is advised that the strain gauge is warmed-up for one hour prior to data collection to maximally improve its reproducibility. In summary, the pressure measuring system shows overall acceptable consistency and reproducibility of test re-test results during equipment warm-up.

4.4.1.4 Study 4: Reproducibility of daily pressure measurements

The study assessed reproducibility of one daily pressure recording over five different days since measurements would be recorded on different days for subjects. The system was set-up in-shoe, switched on and allowed to warm for one hour. The procedure was repeated once daily for five consecutive days for loading and unloading. The equipment was then dismantled and re-assembled and the study repeated. The results over the five days were then compared for reproducibility.

	Coefficient of Variation
BEFORE TRANSPORTATION	
Loading	1.68%
Unloading	1.70%
AFTER TRANSPORTATION	
Loading	1.62%
Unloading	1.64%

Table 4-13: The effects of day to day variation on typical errors produced by the pressure system for loading and unloading, before and after transportation are shown. The values are in kPa.

Table 4-13 shows the effects of day-to-day variation on pressure measurements using the strain gauge. The typical error of pressure measurements, both before and after is low ranging from 1.62% to 1.70%, suggesting a high level of reproducibility.

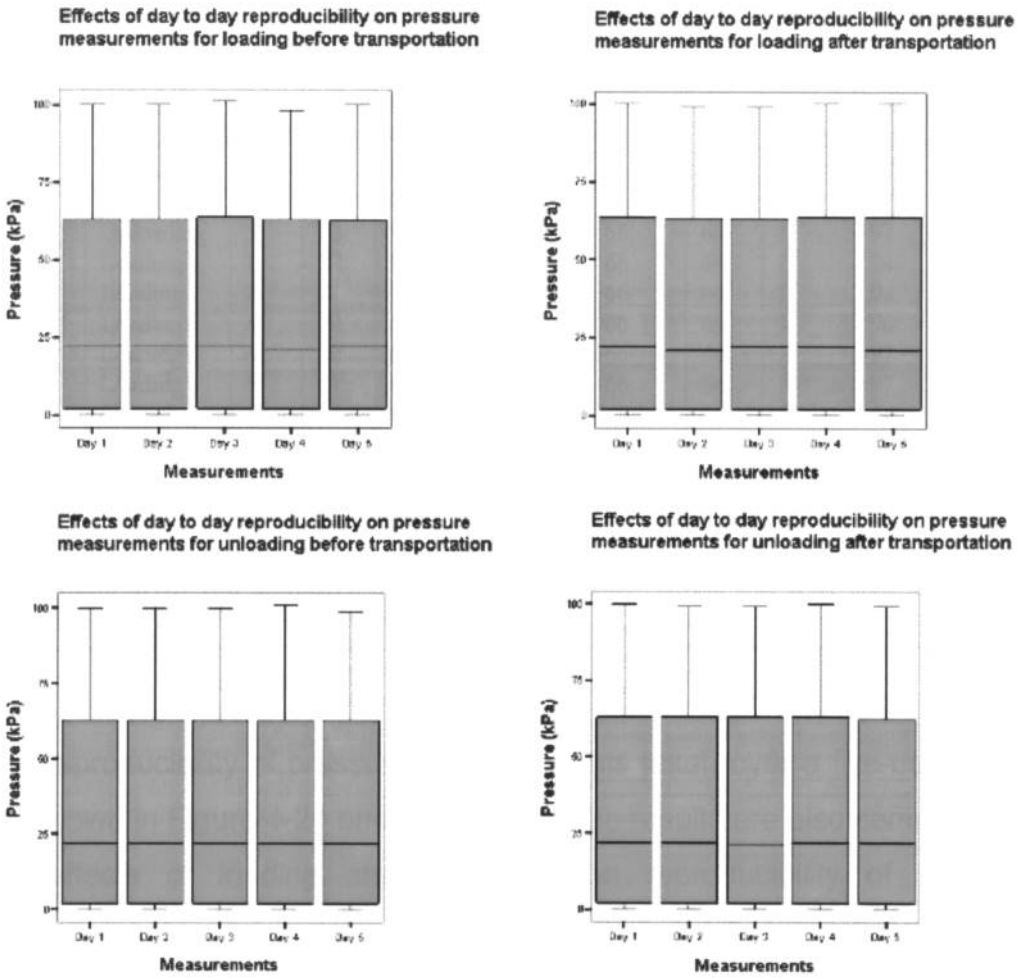


Figure 4-21: Boxplots for loading and unloading, before and after transportation are shown for the effects of day to day variation on pressure measurements using the strain gauge over 5 days.

Before or after transport	Loading or unloading	Measuring day	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentiles 5th	Percentiles 95th	90% Range
Before	Loading	1	22	0	100	2	65	63	1	97	96
Before	Loading	2	22	0	100	2	66	64	1	96	95
Before	Loading	3	22	0	101	2	66	64	1	97	96
Before	Loading	4	21	0	98	2	66	64	1	94	93
Before	Loading	5	22	0	100	2	66	64	1	94	93
Before	Unloading	1	22	0	100	2	66	64	1	94	93
Before	Unloading	2	22	0	100	2	66	64	1	96	95
Before	Unloading	3	22	0	100	2	65	63	1	95	94
Before	Unloading	4	22	0	101	2	65	63	1	97	96
Before	Unloading	5	22	0	99	2	65	63	1	97	96
After	Loading	1	22	0	100	2	66	64	1	94	93
After	Loading	2	21	0	99	2	66	64	1	94	93
After	Loading	3	22	0	99	2	66	64	1	96	95
After	Loading	4	22	0	100	2	66	64	1	97	96
After	Loading	5	21	0	100	2	66	64	1	97	96
After	Unloading	1	22	0	100	2	66	64	1	94	93
After	Unloading	2	22	0	99	2	66	64	1	96	95
After	Unloading	3	21	0	99	2	65	63	1	95	94
After	Unloading	4	22	0	100	2	66	64	1	96	95
After	Unloading	5	22	0	99	2	65	63	1	96	95

Table 4-14: The summary of results for all tests carried out over five days. All pressure values are in kilo-Pascals.

The reproducibility of pressure measurements taken over a five-day period are shown in Figure 4-21 and Table 4-14. The results are also compared for the effects of loading and unloading on reproducibility of pressure measurements. The boxplots above (see Figure 4-21) illustrate the effects of day-to-day variation of measuring pressure using the strain gauge. The x-axis shows the measurements taken from day 1 to 5 and the y-axis represents the measured pressure. The boxplots on the left illustrates results before disassembly, transportation and re-assembly of equipment and the boxplots on the right after. Table 4-14 shows results for the median, minimum and maximum value, 5^{th}, 25^{th}, 75^{th}, and 75^{th} percentiles, the interquartile and 90% ranges.

Close median values are shown on the boxplots of the pressure measurements over five days. These median values ranged between 21 to 22 kPa for all loading and unloading pressure measurements before and after. The interquartile ranges were 63 or 64 kPa and 90% ranges were

between 93 to 96 kPa. Overall, the boxplots, median values and ranges indicate that the reproducibility of pressure measurements over five days is consistent, with test re-test highly reproducible on different days.

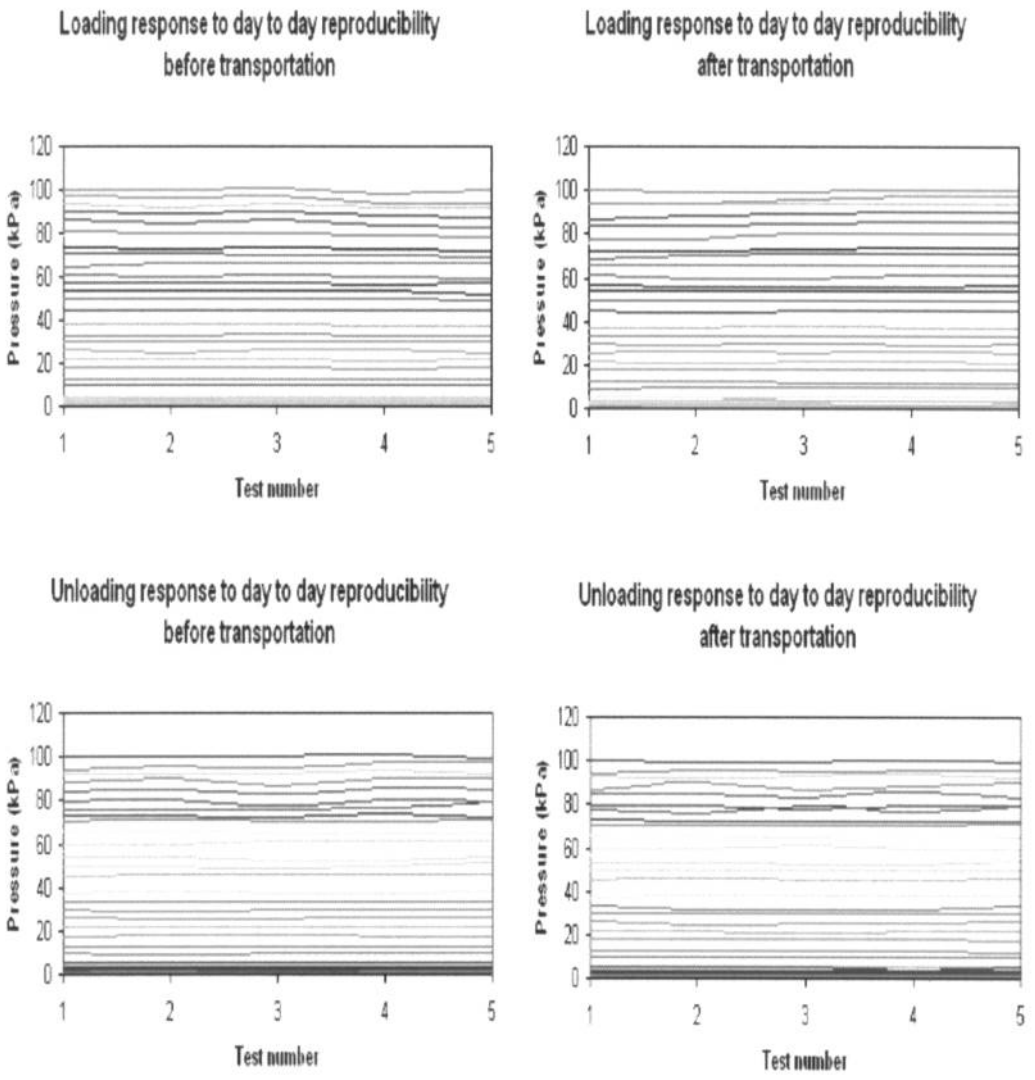


Figure 4-22: Line graphs representative of 5 test sessions repeated over a five-day period. Test number 1 to 5 represent data collected from day 1 to 5.

The reproducibility of individual results was investigated using line graphs (see Figure 4-22). The x-axis represents the test re-test sessions with test numbers 1 to 5 representing the pressure measured on days 1 to 5. The y-axis represents the measured pressure. The top two graphs represents the loading response and the bottom two the unloading response. The left two graphs illustrate the effects of day to day reproducibility before and the right two after reassembly. Overall, the line graphs indicate consistency in reproducibility of pressure measurements on different days.

Further analysis was carried out (see Appendix 4) and pointed to a good level of reproducibility over measurements taken on different days. In summary, the pressure measuring system shows overall acceptable consistency and reproducibility of test re-test pressure values on different days.

4.4.1.5 Study 5: Hysteresis effects on pressure measurements

Measuring instruments produce different outputs or a measurement error for the same value of the same applied quantity according to whether that measurement value was reached by a continuously increasing change (for example, loading pressure on the strain gauge) or a continuously decreasing change (for example, unloading pressure on the strain gauge). This is termed the hysteresis of the measuring instrument and is calculated by dividing the maximum hysteresis (that is, the maximum value for loading and unloading along the y-axis) by the tested range and multiplied by 100 to produce a percentage. The hysteresis for the strain gauge over the tested range of 0 to 100 kPa was ±0.73% and is approximately the same as that stated by its manufacturer (that is, ±1%).

4.4.1.6 Study 6: Range, limit of detection and limit of quantification of pressure measurements

The range of the pressure system is dependant on the range of pressures tested that is 0 to 100 kPa. The limit of detection is the smallest measurement that the system can detect and was carried out by applying unquantifiable pressure on the strain gauge and recording the output. The limit of detection is the lowest measurement that the system can record above zero that can be quantified with defined accuracy under experimental conditions. The range of the pressure system is 0 to 100 kPa. The limit of detection is 0.7 kPa and the limit of quantification is 0.8 kPa.

4.4.2 Validation studies of the laser Doppler fluxmeter

The laser Doppler fluxmeter system has been extensively validated by a number of authors and is an established method to measure skin blood flow (discussed in Section 2.1.6). However, it is vital that researchers validate any equipment used to exclude any errors produced by their own system due to wear and tear or damage that may not be visible but that might affect

the performance of the equipment and lead to misleading conclusions. Thus, the laser Doppler fluxmeter system was tested for the same engineering parameters that the strain gauge was tested.

4.4.2.1 Study 7: Linearity of laser Doppler fluxmeter measurements

An *in vitro* study was carried out with the aim of investigating whether laser Doppler fluxmeter measurements produced a linear response to increases in blood speed. An *in vitro* study was chosen because controlling the speed of blood flow *in vivo* is difficult and specialised equipment was not available. Room temperature was controlled at 23.7°C ±0.2°C. The temperature at the laser Doppler fluxmeter probe tip was also monitored throughout the experiment and was 23.4°C ±0.8°C.

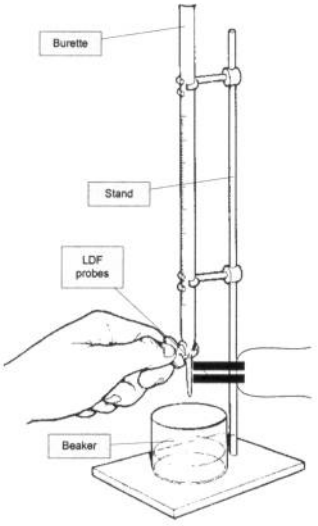


Figure 4-23: Apparatus used for the *in vivo* study using porcine blood.

Heparin, 1 millilitre to every 100 millilitres of blood, was added to prevent the pig's blood from coagulating and the above apparatus set-up (see Figure 4-23). Blood volume speed was calculated by the amount of blood that had flowed past the transducers into a beaker over a given period of time.

Linearity of experimental *in vitro* model measurements for blood speed against the laser Doppler fluxmeter DRT4 blood cells speed output was recorded. The x-axis on the scatterplot (see Figure 4-24) shows the laser Doppler fluxmeter blood cell speed and the y-axis the *in vitro* model blood volume speed. The scatterplot below shows a fair level of correlation

despite the model measuring blood volume speed and the laser Doppler fluxmeter measuring average blood cell speed. Similarly, the proportion of variance showed a fair level of correlation with an R^2 value of 0.75, thus, the laser Doppler fluxmeter's output accounts for 75% of the *in vitro* model's measured value.

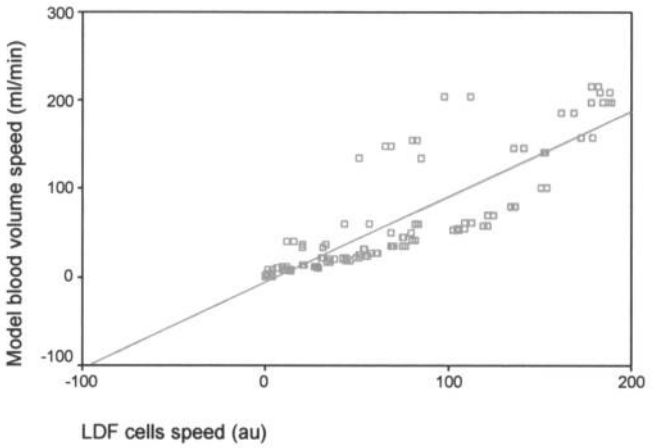


Figure 4-24: Scatterplot of the model's blood volume speed against the laser Doppler fluxmeter DRT4's blood cells speed. The linear regression line is also shown.

A regression ANOVA was carried out suggesting linearity with a value of $p<0.01$. The x-axis on the scatterplot (see Figure 4-25) shows the regression standardised predicted values and the y-axis the regression standardised residuals values. The scatterplot below for the regression standardised residual against the regression standardised predicted value show some possible bias in the assumption of linearity and homogeneity of variance, however, this may be due to the fact that the blood flow model used and the laser Doppler fluxmeter were measuring difference variables of blood speed.

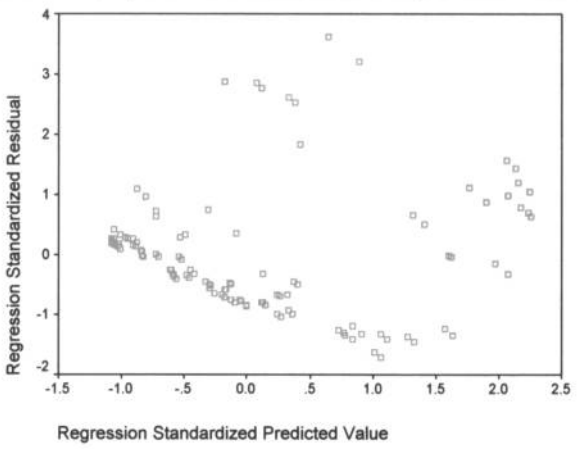


Figure 4-25: Scatterplot for blood volume speed to test assumptions of linearity and homogeneity of variance.

4.4.2.2 Study 8: Accuracy of laser Doppler fluxmeter measurements

Following the calibration method described in section 4.3.1 for the laser Doppler fluxmeter, the accuracy of the system was tested against the standard for repeated measures to ensure that the system was functioning correctly. Using Brownian motion of a known concentration of latex spheres in a suspension, repeated measurements of calibration fluid were taken under experimental conditions and the equipment's error calculated. Room temperature was kept at 20.8°C ±0.7°C.

The ruggedness of the equipment was checked by dissembling the equipment, packing it, transporting it for approximately one hour, unpacking and re-assembling the laser Doppler fluxmeter system. Following reassembling the above procedure using Brownian motion was repeated to test for accuracy of measurements. Room temperature was maintained at 20.9°C ±0.7°C.

Bland/Altman plots were constructed to test the accuracy of measured flux against known flux values for calibration fluid (see Figure 4-26, Figure 4-27 and Figure 4-28). For the Bland/Altman plot and the calculation of the coefficient of repeatability the standard value of 210 AU for the known flux value was used. In all three Bland/Altman plots for flux the x-axis represents the mean standard and measured flux and the y-axis the difference between the standard and measured flux. Before transportation the limits of

agreement were 0.58 above (AU) above and –3.40 (AU) below the mean difference for measured and known flux. After dismantling, transporting and reassembly the limits of agreement were 0.00 AU and –3.93 AU above and below the mean difference. Overall, the limits of agreement for this study were 0.35 AU above and –3.72 AU below the mean difference of –1.69 AU. These limits of agreement are within the range for accuracy set by the manufacturers of the laser Doppler fluxmeter DRT4 (that is, ± 20 AU) and show a high degree of accuracy for the measurement of flux.

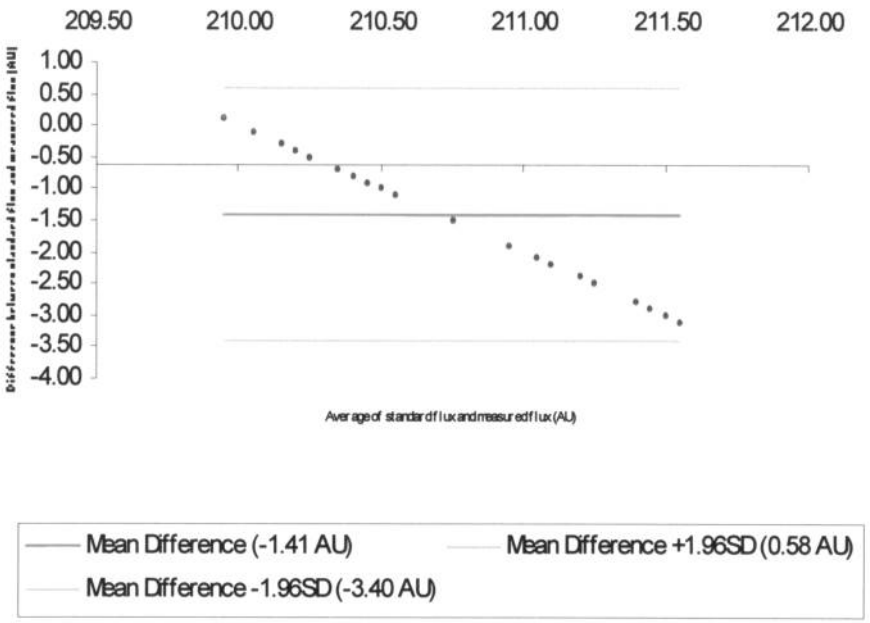


Figure 4-26: Bland/Altman plot for the accuracy of measured flux values using the laser Doppler fluxmeter DRT4 against known values for calibration fluid (that is, 210±20) before dismantling, transportation and reassembly.

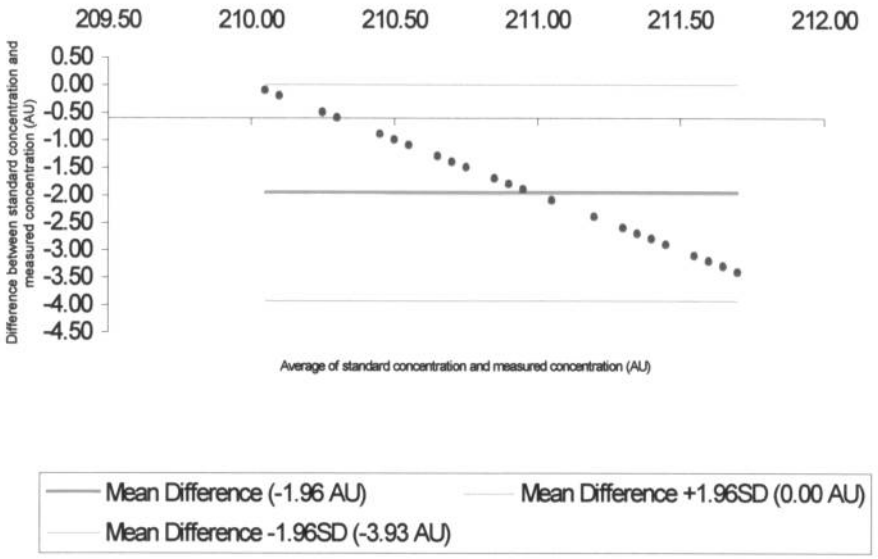


Figure 4-27: Bland/Altman plot for the accuracy of measured flux values using the laser Doppler fluxmeter DRT4 against known values for calibration fluid (that is, 210±20) after dismantling, transportation and reassembly.

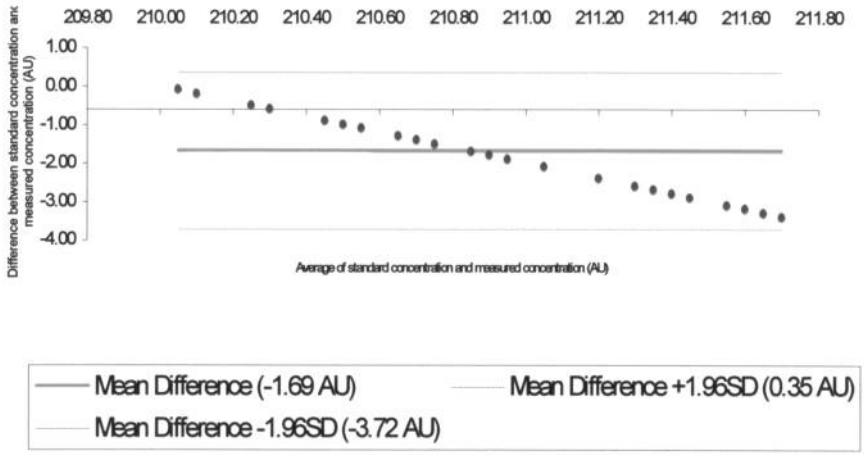


Figure 4-28: Bland/Altman plot for the accuracy of all measured flux values using the laser Doppler fluxmeter DRT4 against known values for calibration fluid (that is, 210±20) for all data.

To test the accuracy of measured concentration of latex spheres in calibration fluid against the known concentration values three Bland/Altman plots were constructed (see Figure 4-29, Figure 4-30 and Figure 4-31). For the Bland/Altman plot and the calculation of the coefficient of repeatability the standard value of 240 AU for the known concentration of latex spheres was used. For the three Bland/Altman plots below the x-axis represents the mean standard and measured concentration of latex spheres and the y-axis the difference between the standard and measured concentration of latex spheres. Before transportation the limits of agreement for this study were

3.09 AU above and 0.88 AU below the mean difference for measured and known concentration of latex spheres. After dismantling, transporting and reassembly of the laser Doppler fluxmeter DRT4 the limits of agreement were 3.44 AU above and 0.85 below the mean difference. Comparing the before and after transportation values the results indicate that the accuracy of the equipment tested is not affected by relocating. The overall limits of agreement the measurement of concentration was 3.27 AU above and 0.86 AU below the mean difference indicating that the laser Doppler fluxmeter DRT4 tested produces highly accurate results within the limits set by the manufacturer's (that is, ± 20 AU).

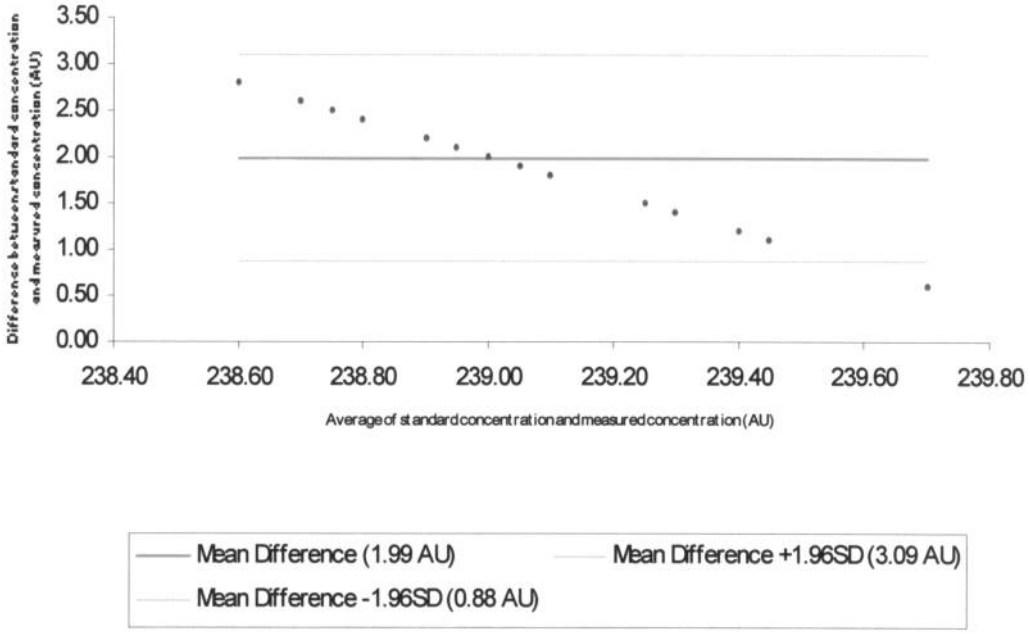


Figure 4-29: Bland/Altman plot for the accuracy of measured concentration of latex spheres values using the laser Doppler fluxmeter DRT4 against known values for calibration fluid (that is, 240±20) before dismantling, transportation and reassembly.

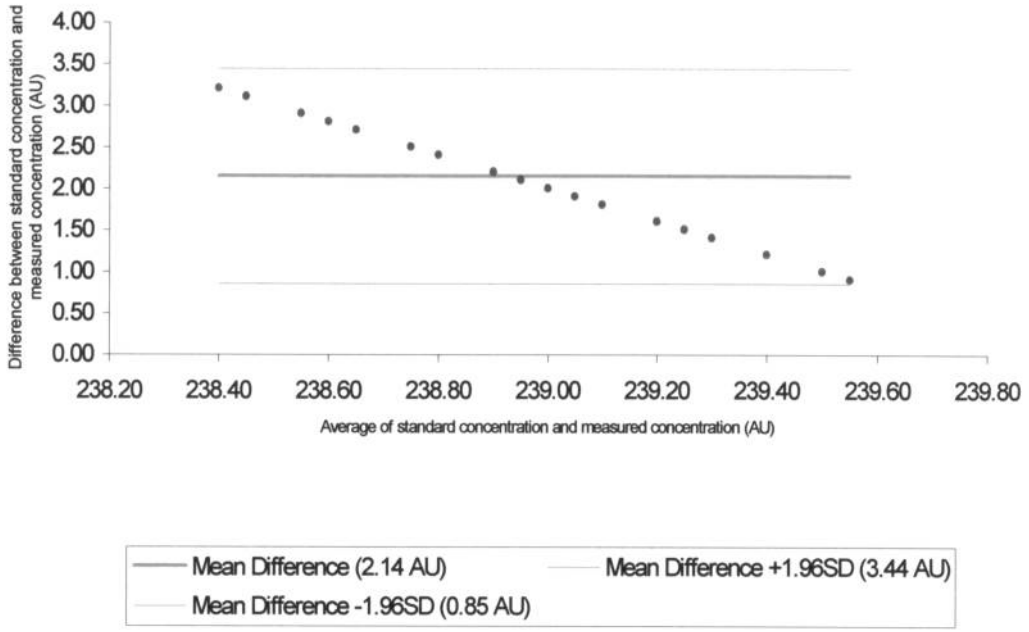


Figure 4-30: Bland/Altman plot for the accuracy of measured concentration of latex spheres values using the laser Doppler fluxmeter DRT4 against known values for calibration fluid (that is, 240±20) after dismantling, transportation and reassembly.

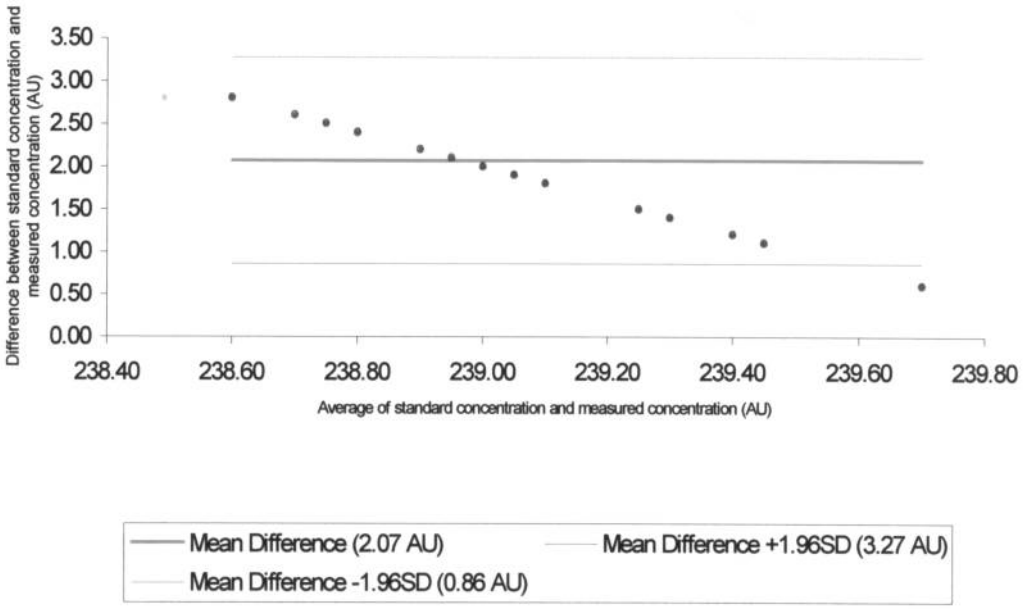


Figure 4-31: Bland/Altman plot for the accuracy of all measured concentration of latex spheres values using the laser Doppler fluxmeter DRT4 against known values for calibration fluid (that is, 240±20) for all data.

For all the Bland/Altman plots the correlation coefficient was carried out to determine any associations between the average of the standard and measured flux or concentrations and the difference between the two variables measured for flux and concentration before, after and for all the

data collected (see Table 4-15). All the Bland/Altman plots showed a strong negative correlation between the difference of the standard values and the measured values against the average of the standard values and the measured values. This may have resulted as a result of the room and calibration fluid cooling during the experiment, hence a negative correlation as Brownian motion of the latex spheres within the solution decreased in motion with decreasing room and fluid temperature.

	Correlation correlation	***Correlation significance***
FLUX		
Before transportation	r = -1	P<0.01
After transportation	r = -1	P<0.01
All data	r = -1	P<0.01
CONCENTRATION		
Before transportation	r = -1	P<0.01
After transportation	r = -1	P<0.01
All data	r = -1	P<0.01

Table 4-15: Correlation coefficient for all the Bland/Altman plots for the accuracy of the laser Doppler fluxmeter measurements.

	Coefficient of repeatability
FLUX	
Before transportation	1.99
After transportation	1.96
All data	2.03
CONCENTRATION	
Before transportation	1.11
After transportation	1.30
All data	1.20

Table 4-16: The coefficient of repeatability (accuracy) for flux and concentration before and after dismantling, transporting and reassembling the equipment.

The above table illustrates the accuracy of the laser Doppler fluxmeter for measuring flux and concentration. At a 95% confidence interval the coefficient of repeatability (accuracy) is ±1.99 AU before ±1.96 AU after transporting for flux, indicating as previously mentioned that transferring equipment to a new location does not affect it. The overall accuracy for measured flux is ±2.03 AU. The tested laser Doppler fluxmeter DRT4 shows a high level of accuracy and is within the manufacturers recommendations and demonstrates correct operation.

Similarly, before transporting and at a 95% confidence interval the coefficient of repeatability (accuracy) is ±1.11 AU for concentration of latex spheres in calibration fluid. Following transportation the accuracy is minimally affected with a coefficient of repeatability of ±1.30 AU. The overall accuracy for the test equipment for concentration is ±1.20 AU. Since the accuracy value for the tested laser Doppler fluxmeter unit is below the limits set by the manufacturer it indicates correct operation of the system to measure concentration.

4.4.2.3 Study 9 and 10: Repeatability of laser Doppler fluxmeter measurements

An *in vitro* and an *in vivo* study was carried out with the aim of determining the repeatability of the blood flow measuring system under controlled experimental conditions. The *in vitro* study consisted of taking repeated measurements of calibration fluid at a room temperature of 20.8°C ±0.7°C and comparing the results for flux and concentration.

The *in vivo* study consisted of taking repeated measurements of skin blood flow using one healthy subject. Due to variability between physiological states between subjects only one subject was selected for this study. Room temperature was maintained at 21.4°C ±0.7°C. The general exclusion criteria and measures to reduce experimental errors described in Appendix 1 were followed. The in-shoe measuring system was set-up as mentioned in section 4.2.6 and the method described to collect semi-weight bearing data used. One foot was selected at random for all the measurements. Skin blood flow was then recorded at the centre of the heel. The foot was then taken out of the shoe-device and the above procedure repeated a number of times.

The ruggedness of the equipment was also tested. Room temperature was maintained at 20.7°C ±0.7°C for the *in vitro* study and 21.1°C ±0.6°C for the *in vivo* study.

4.5.2.3.1 Study 9: In vitro laser Doppler fluxmeter repeatability

	Coefficient of Variation
FLUX	
Before transportation	0.48%
After transportation	0.47%
CONCENTRATION	
Before transportation	0.35%
After transportation	0.36%

Table 4-17: The effects of test re-test on typical errors produced by the laser Doppler fluxmeter for flux and concentration, before and after transportation are shown. The values are in arbitrary units (AU).

Table 4-17 shows the typical errors due to test re-test of laser Doppler fluxmeter measurements for flux and concentration. All the values for the coefficient of variation are small indicating a high level of repeatability in *in vitro* measurements.

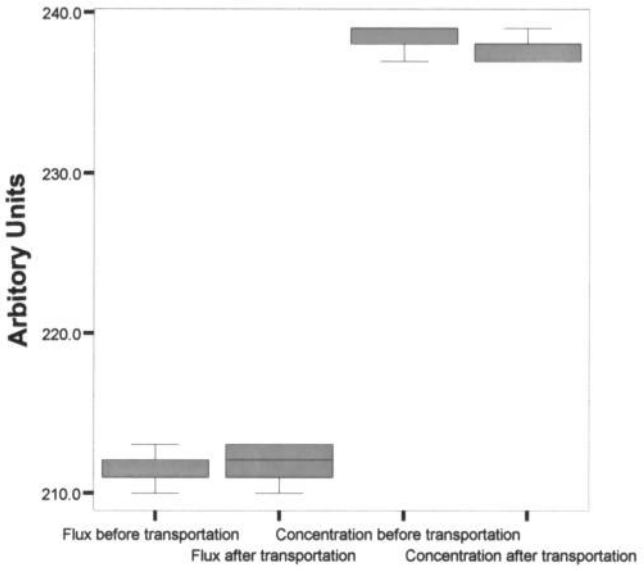


Figure 4-32: The repeatability of the laser Doppler fluxmeter for flux and concentration before and after transportation.

Before or after transport	Flux or concentration	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentiles 5th	Percentiles 95th	90% Range
Before	Flux	211	210	213	211	212	1	210	213	3
After	Flux	212	210	213	211	213	2	210	213	3
Before	Concentration	238	237	239	238	239	1	237	239	2
After	Concentration	238	237	239	237	238	1	237	239	2

Table 4-18: Summary of repeatability results for the test re-tests of flux and concentration measurements using calibration fluid and the laser Doppler fluxmeter DRT4. A total of 29 repeated measures were taken for the flux and concentration before and after transportation. All results are in arbitrary units.

The boxplot (see Figure 4-32) for flux and concentration measurements for test re-test taken before and after moving the skin blood flow equipment indicates a high degree of repeatability. The x-axis shows the measurement sessions of flux and concentration for before and after. The y-axis shows the laser Doppler fluxmeter perfusion arbitrary units of measures. The medians of flux before and after dismantling, transportation and reassembly of equipment are 211 and 212 AU respectively (see Table 4-18) indicating closeness of the agreement. Similarly, the measurement of concentration shows closeness of agreement with median values of 238 AU for both measurements. The 90% range, interquartile range, percentiles presented and the maximum and minimum values in the above table confirms the closeness of agreement and consistency of repeated measurements taken before and after relocating the equipment.

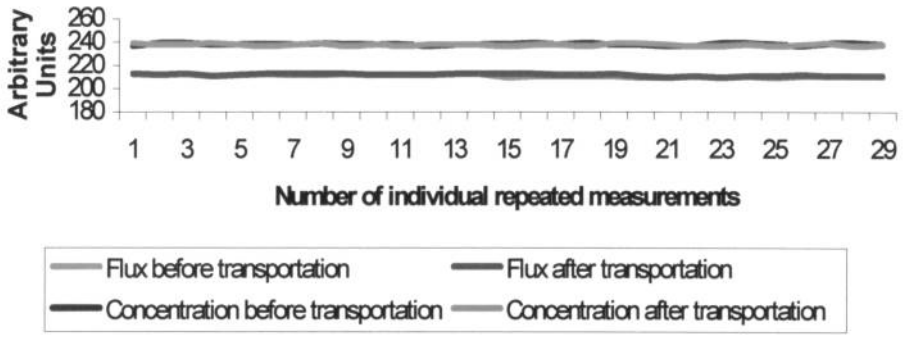


Figure 4-33: Line graphs for the individual 39 repeated measurements of flux and concentration for before and after dismantling, transportation and reassembly.

To investigate the repeatability of the individual measurements taken for flux and concentration for ruggedness of equipment a line graph was constructed (see Figure 4-33). The x-axis represents the number of individual test re-test measurements taken, that is, 29 repeated measurements for each of the variables shown in the line chart above. The y-axis shows the perfusion arbitrary units measured by the laser Doppler fluxmeter. Overall, the line graphs indicate consistency in repeatability of repeated measures of flux and concentration taken before and after reassembly of the equipment.

No of measurements	Variable	Median	Minimum	Maximum	Percentiles		IQR	Percentiles		90% range
					25th	75th		5th	95th	
29	Flux	211	210	213	211	212	1	210	213	3
29	Concentration	238	237	239	238	239	1	237	239	2

Table 4-19: The analysis of within measurements for the *in vitro* repeatability experiment before dismantling, transportation and reassembly of the equipment. The analysis relates to 29 repeated measurements. All data is in perfusion Arbitrary Units.

Further analysis of medians, minimum and maximum values, the 5th, 25th, 75th and 90th percentile and the interquartile and 90% range was carried out (see Table 4-19 and Table 4-20). The small ranges and closeness between the minimum and maximum values for flux and concentration indicates a high degree of repeatability within the repeated measurements for before and after relocation of the equipment using an *in vitro* model.

No of measurements	Variable	Median	Minimum	Maximum	Percentiles		IQR	Percentiles		90% range
					25^{th}	75^{th}		5^{th}	95^{th}	
29	Flux	212	210	213	211	213	2	210	213	3
29	Concentration	238	237	239	237	238	1	237	239	2

Table 4-20: The analysis of within measurements for the *in vitro* repeatability experiment after dismantling, transportation and reassembly of the equipment. The analysis relates to 29 repeated measurements. All data is in perfusion Arbitrary Units.

4.5.2.3.2 Study 10: In vivo laser Doppler fluxmeter repeatability

	Coefficient of Variation
FLUX	
Before transportation	11.77%
After transportation	12.22%
CONCENTRATION	
Before transportation	2.71%
After transportation	2.74%
SPEED	
Before transportation	20.06%
After transportation	21.28%

Table 4-21: The *in vivo* effects of test re-test on typical errors produced by the laser Doppler fluxmeter for flux, concentration and speed, before and after transportation are shown. The values are in arbitrary units (AU).

Table 4-21 illustrates the *in vivo* typical errors that occur after repeated measurements. The variance for blood cell speed is high and for the concentration of cells is low. The calculation of flux is related to the values for concentration and speed and its high values for both before and after transportation reflects the high values measured for blood cell speed. When compared to the previous experiment the typical errors for the *in vivo* study are much greater than the *in vitro* study indicating the complexity of controlling the random *in vivo* physiological responses.

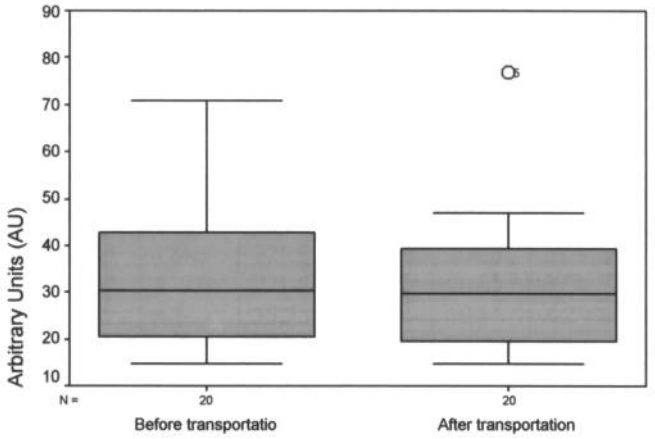


Figure 4-34: *In vivo* repeatability of laser Doppler fluxmeter measurements for blood cell flux before and after dismantling, transporting and reassembling the equipment.

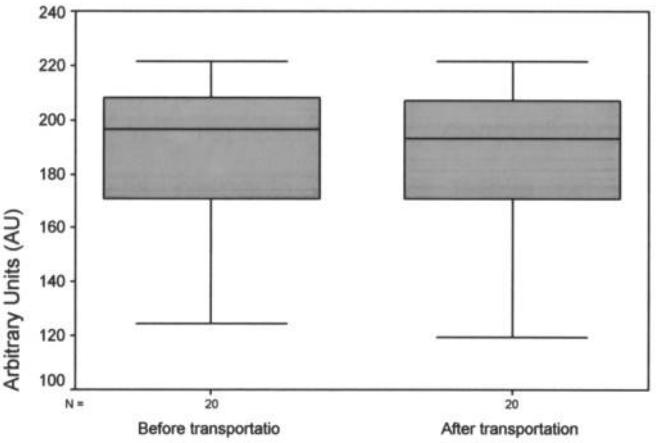


Figure 4-35: *In vivo* repeatability of laser Doppler fluxmeter measurements for concentration of cells before and after dismantling, transporting and reassembling the equipment.

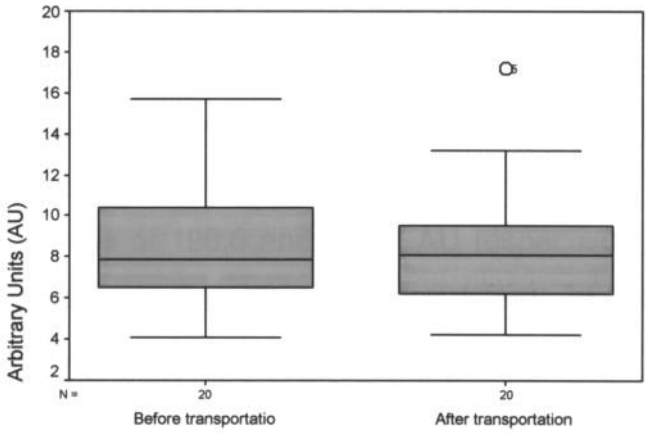


Figure 4-36: *In vivo* repeatability of laser Doppler fluxmeter measurements for speed of cells before and after dismantling, transporting and reassembling the equipment.

Before or after transport	Flux or concentration	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentiles 5th	Percentiles 95th	90% Range
Before	Flux	30.3	14.7	70.8	18.5	43.0	24.5	14.7	69.6	54.9
After	Flux	29.9	14.7	76.9	17.9	39.5	21.6	14.7	75.4	60.7
Before	Concentration	196.6	124.8	221.5	171.1	208.6	37.5	125.8	221.3	95.5
After	Concentration	192.9	119.6	221.6	169.5	207.1	37.6	120.8	221.3	100.5
Before	Speed	7.9	4.1	15.7	6.3	10.5	4.2	4.1	15.5	11.4
After	Speed	8.1	4.2	17.2	6.2	9.6	3.4	4.2	17.0	12.8

Table 4-22: Summary of *in vivo* repeatability results for test re-tests of flux, concentration and speed of moving blood cells measurements using the laser Doppler fluxmeter DRT4. A total of 20 repeated measures were taken for the flux, concentration and speed before and after transportation. All results are in arbitrary units.

Boxplots were constructed for flux, concentration and speed of blood cells for before and after (see Figure 4-34, Figure 4-35 and Figure 4-36). For all three boxplots, the x-axis represents flux, concentration and speed of moving red blood cells respectively. The y-axis on all the boxplots represents arbitrary units (AU). Figure 4-34 and Figure 4-36 show one outlier each, 1.5 of the interquartile range, both for after dismantling and relocation of the laser Doppler fluxmeter DRT4. All the boxplots show a good level of consistency of test re-test. Table 4-22 confirms the findings of the boxplots. For blood cell flux, the medians indicate closeness of agreement for before and after transportation with values for 30.3 and 29.9 AU respectively. The 90% and interquartile ranges, percentiles, minimum and maximum values indicate closeness of agreement and consistency of repeated blood cell flux measurements for all the data collected. For concentration of blood cells, the medians also show closeness of agreement with values of 196.6 and 192.9 AU respectively for before and after moving equipment. For all the concentration of blood cells data presented, the ranges, percentiles, minimum and maximum values show closeness of agreement and consistency of repeated measurements. Finally, the speed of blood cells show similar trends with closeness of agreement and a high degree of repeatability with medians of 7.9 and 8.1 AU for before and after respectively, and close ranges, percentiles and minimum and maximum values.

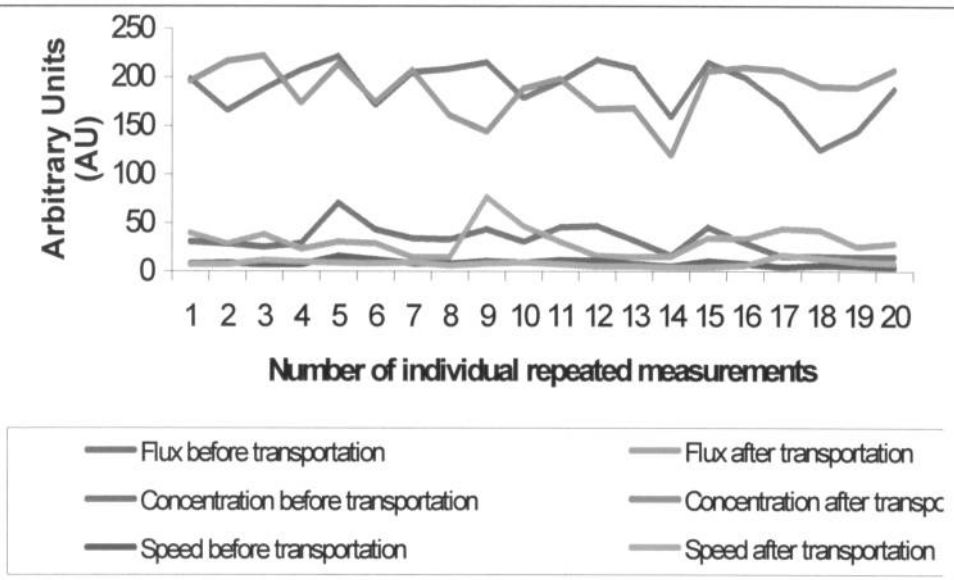


Figure 4-37: Line graph for the individual 20 repeated *in vivo* repeated measurements of blood cell flux, concentration and speed for before and after dismantling, transportation and reassembly.

The repeatability of individual measurements of blood cell flux, concentration and speed, for before and after was carried out by constructing a line graph (see Figure 4-37). The x-axis represents the number of individual measurements taken, that is, 20 for each of the 6 variables presented. The y-axis shows the perfusion arbitrary units. Some level of consistency is shown for flux, concentration and speed of blood cells. Overall, the line graph illustrates the physiological factors that affect the repeatability of results *in vivo* for each individual repeated measurement taken. However, within the study limits the level of repeatability error is acceptable.

No of measurements	Variable	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentiles 5th	Percentiles 95th	90% range
20	Flux	30	15	71	23	43	20	15	48	33
20	Concentration	197	125	222	171	208	37	143	218	75
20	Speed	8	4	16	7	10	4	5	12	8

Table 4-23: The analysis of within measurements for the *in vivo* repeatability experiment before dismantling, transportation and reassembly of the equipment. The analysis relates to 20 repeated measurements. All data is in perfusion Arbitrary Units.

Considering the physiological variability that exists when measuring within sites and between subjects, the investigation of median, minimum and maximum values, 5th, 25th, 75th and 95th percentiles and interquartile and 90% range showed a good level of repeatability with higher ranges shown by concentration rather than flux or speed (see Table 4-23 and Table 4-24). Since the *in vitro* study showed significantly less variability for the within measurements the variability shown in this study appears to be related to human variability of local skin blood flow rather than equipment errors.

No of measurements	Variable	Median	Minimum	Maximum	Percentiles		IQR	Percentiles		90% range
					25th	75th		5th	95th	
20	Flux	30	15	77	22	39	17	15	48	34
20	Concentration	193	120	222	172	207	35	142	217	74
20	Speed	8	4	17	6	9	3	4	13	9

Table 4-24: The analysis of within measurements for the *in vivo* repeatability experiment after dismantling, transportation and reassembly of the equipment. The analysis relates to 20 repeated measurements. All data is in perfusion Arbitrary Units.

4.4.2.4 Study 11: Reproducibility of measurements during equipment warm-up

The reproducibility of the laser Doppler fluxmeter's measurements during equipment warm-up was tested using Brownian motion and calibration fluid. Room temperature was kept at 20.6°C ±0.6°C. The laser Doppler fluxmeter was set-up as for calibration. The probes were inserted in the fluid and the equipment switched on and a baseline measurement taken. The equipment was left with the power on and subsequent measurements taken at one, two and three hour intervals from baseline. The effects of equipment warm-up on the reproducibility of results were calculated.

The ruggedness of equipment warm-up on the reproducibility of results was tested by following the above method, after dismantling, transportation and reassembly of the equipment. Room temperature was maintained at 20.9°C ±0.7°C.

	Coefficient of Variation
FLUX	
Before transportation	0.42%
After transportation	0.40%
CONCENTRATION	
Before transportation	0.27%
After transportation	0.29%

Table 4-25: The *in vitro* effects of equipment warm-up on typical errors produced by the laser Doppler fluxmeter for flux and concentration, before and after transportation are shown. The values are in arbitrary units (AU).

The effects of equipment warm-up on typical errors generated by the laser Doppler fluxmeter DRT4 are shown in Table 4-25 for flux and concentration of latex spheres suspended in calibration fluid. The typical errors for reproducibility of the equipment are very small suggesting that equipment warm-up has very little effect on repeated measurements.

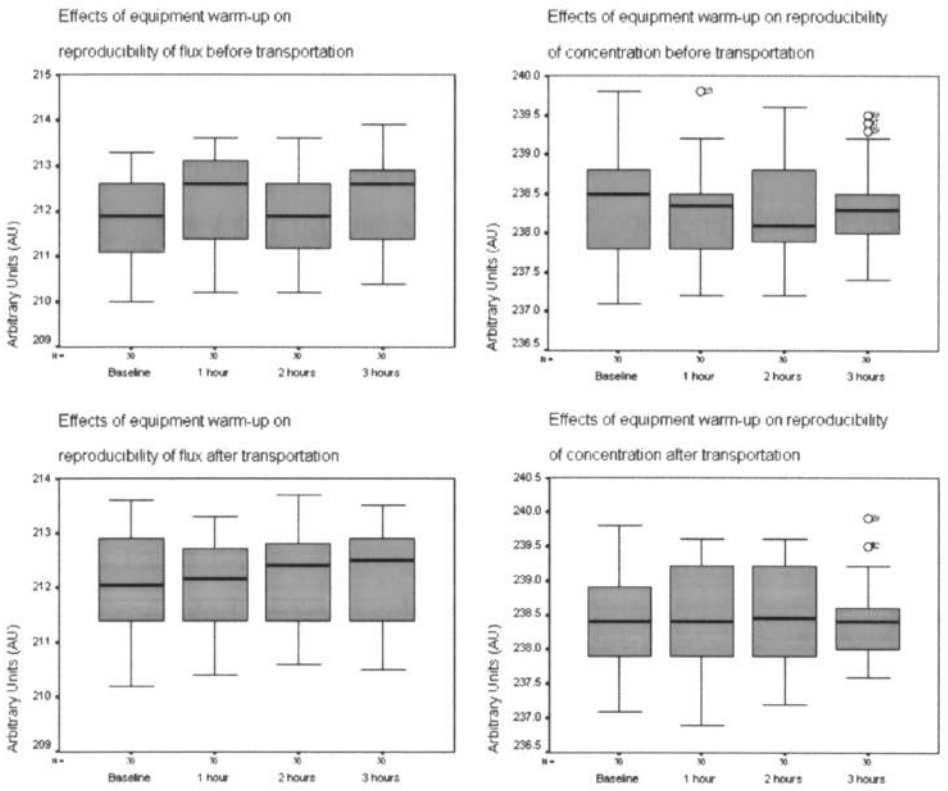


Figure 4-38: Effects of equipment warm-up on reproducibility of flux and concentration values for before and after dismantling, transportation and reassembly of the laser Doppler fluxmeter unit.

Before or after transport	Flux or concentration	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentiles 5th	Percentiles 95th	90% Range
Before	Flux (baseline)	211.9	210.0	213.3	211.1	212.6	1.5	210.4	213.3	2.9
Before	Flux (1 hour)	212.6	210.2	213.6	211.4	213.1	1.7	210.5	213.4	2.9
Before	Flux (2 hours)	211.9	210.2	213.6	211.2	212.6	1.4	210.6	213.6	3
Before	Flux (3 hours)	212.6	210.4	213.9	211.4	212.9	1.5	210.5	213.8	3.3
After	Flux (baseline)	212.1	210.2	213.6	211.4	212.9	1.5	210.6	213.5	2.9
After	Flux (1 hour)	212.2	210.4	213.3	211.4	212.7	1.3	210.6	213.3	2.7
After	Flux (2 hours)	212.4	210.6	213.7	211.4	212.8	1.4	210.6	213.6	3
After	Flux (3 hours)	212.5	210.5	213.5	211.4	212.9	1.5	210.6	213.5	2.9
Before	Conc. (Baseline)	238.5	237.1	239.8	237.8	238.8	1	237.1	239.6	2.5
Before	Conc. (1 hour)	238.4	237.2	239.2	237.8	238.5	0.7	237.3	239.2	1.9
Before	Conc. (2 hours)	238.1	237.2	239.6	237.9	238.8	0.9	237.2	239.6	2.4
Before	Conc. (3 hours)	238.3	237.4	239.5	238.0	238.5	0.5	237.6	239.5	1.9
After	Conc. (Baseline)	238.4	237.1	239.8	237.9	238.9	1	237.2	239.7	2.5
After	Conc. (1 hour)	238.4	236.9	239.6	237.9	239.2	1.3	237.3	239.6	2.3
After	Conc. (2 hours)	238.5	237.2	239.6	237.9	239.2	1.3	237.2	239.6	2.4
After	Conc. (3 hours)	238.4	237.6	239.9	238.0	238.6	0.6	237.8	239.7	1.9

Table 4-26: Summary of equipment warm-up on reproducibility of results for test re-tests of flux and concentration measurements of latex spheres in a suspension using the laser Doppler fluxmeter DRT4. A total of 30 repeated measures were taken for the flux and concentration before and after transportation. All results are in arbitrary units.

The x-axis in Figure 4-38 shows the baseline and measurements taken at 1, 2 and 3 hours from baseline for flux and concentration of laser Doppler fluxmeter measurements. The y-axis shows the perfusion arbitrary units. The above boxplots for reproducibility of test re-test results for flux and concentration for before and after indicate closeness of agreement. The two boxplots for concentration contains some outliers 1.5 times the interquartile. For flux, the medians show consistency with a small range of 211.9 to 212.6 AU (see Table 4-26). All the ranges, percentiles, minimum and maximum values presented indicate closeness of agreement and consistency of reproducibility of results during warm-up of the equipment to measure skin blood flow. For concentration, all the parameters shown in the above table also demonstrates closeness of agreement and consistency of reproducibility of results.

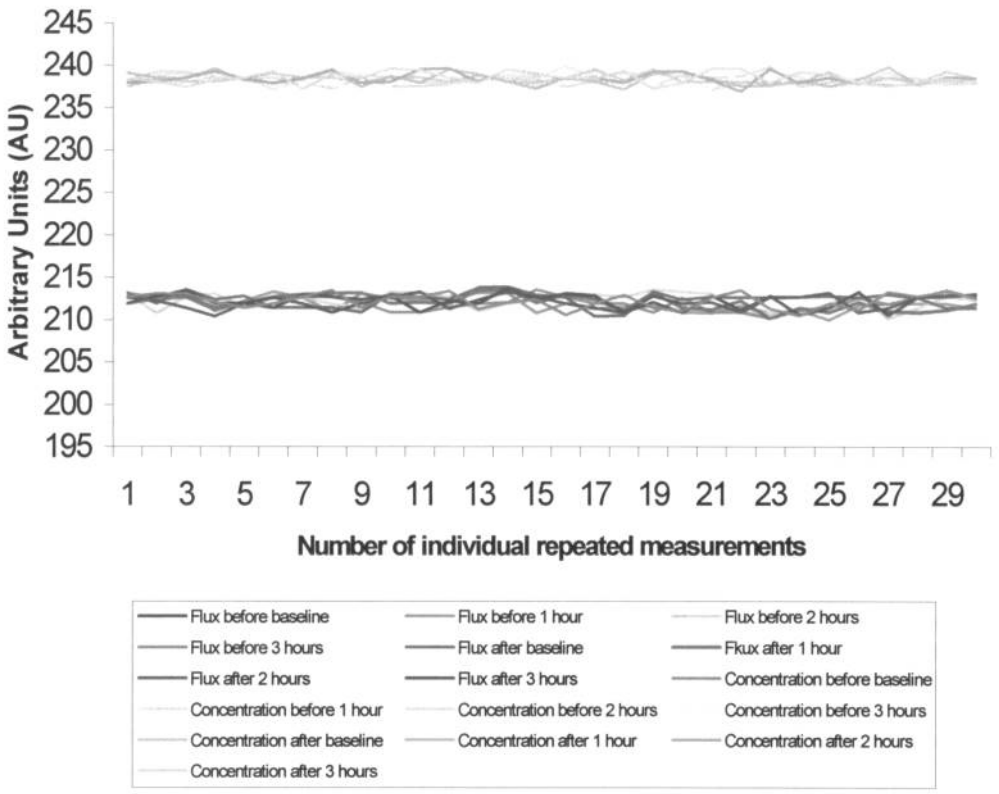


Figure 4-39: Line graph for the individual 30 repeated measurements of flux and concentration for before and after dismantling, transportation and reassembly of the laser Doppler fluxmeter during equipment warm-up.

To investigate the effects of equipment warm-up on the individual measurements taken for flux and concentration before and after dismantling, transportation and reassembly of the laser Doppler fluxmeter DRT4 a line graph was constructed (see Figure 4-39). The x-axis represents the number of individual test re-test measurements taken for each of the variables shown above. Overall, the line graph above indicates consistency in reproducibility of repeated measures taken for flux and concentration before and after relocation of the measuring equipment. The line graph above also indicates no differences between the before and after recordings.

No of measurements	Measurement	Variable	Median	Minimum	Maximum	Percentiles		IQR	Percentiles		90% range
						25th	75th		5th	95th	
30	Baseline	Flux	212	210	213	211	213	1	211	213	2
30	1 hour	Flux	212	210	213	211	213	1	211	213	2
30	2 hours	Flux	213	210	214	211	213	2	211	213	3
30	3 hours	Flux	212	210	214	211	213	1	211	213	2
30	Baseline	Conc.	239	237	240	238	239	1	237	239	2
30	1 hour	Conc.	238	237	240	238	239	1	237	239	2
30	2 hours	Conc.	238	237	240	238	239	1	237	239	2
30	3 hours	Conc.	238	237	240	238	239	1	237	239	2

Table 4-27: The analysis of within measurements for the effects of equipment warm-up before dismantling, transportation and reassembly of the equipment. The analysis relates to 30 repeated measurements carried out 4 times at 1-hour interval. All data is in perfusion Arbitrary Units.

To investigate the effects of equipment warm-up on the reproducibility of results analysis of variability was carried out by calculating the medians, minimum and maximum values, the 5th, 25th, 75th and 90th percentile and the interquartile and 90% range was carried out (see Table 4-27 and Table 4-28). The small ranges and closeness between the minimum and maximum values, with only 1 AU difference between the median and minimum and maximum values for flux and concentration, indicates a high degree of repeatability for equipment warm-up before and after dismantling, transporting and reassembling the equipment using an *in vitro* model.

No of measurements	Measurement	Variable	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentiles 5th	Percentiles 95th	90% range
30	Baseline	Flux	213	210	214	212	213	1	211	214	3
30	1 hour	Flux	212	210	214	211	213	1	211	213	2
30	2 hours	Flux	212	210	213	211	213	1	211	213	2
30	3 hours	Flux	212	211	214	212	213	1	211	213	3
30	Baseline	Conc.	238	237	240	238	239	1	238	239	2
30	1 hour	Conc.	238	237	240	238	239	1	237	240	2
30	2 hours	Conc.	238	237	240	238	239	1	238	240	2
30	3 hours	Conc.	238	237	240	238	239	1	237	240	2

Table 4-28: The analysis of within measurements for the effects of equipment warm-up after dismantling, transportation and reassembly of the equipment. The analysis relates to 30 repeated measurements carried out 4 times at 1-hour interval. All data is in perfusion Arbitrary Units.

4.4.2.5 Study 12: Reproducibility of daily measurements

The procedure explained above for reproducibility of equipment warm-up (see section 4.4.2.4) was repeated but on recordings on five different days to test whether day to day variability affects laser Doppler fluxmeter output. Room temperature was kept at 21.2±0.9°C.

The ruggedness of the equipment in relation to daily recordings was also carried out. Room temperature was maintained at 20.9 ±0.8°C.

	Coefficient of Variation
FLUX	
Before transportation	0.44%
After transportation	0.40%
CONCENTRATION	
Before transportation	0.29%
After transportation	0.27%

Table 4-29: The *in vitro* effects of day to day variation on typical errors produced by the laser Doppler fluxmeter for flux and concentration, before and after transportation are shown. The values are in arbitrary units (AU).

The effects of day-to-day laser Doppler fluxmeter measurements variability for test re-test are shown in Table 4-29 above for flux and concentration

before and after reassembly of equipment over a five-day period. The coefficient of variation values for both flux and concentration are low suggesting that measurements taken on different days are highly repeatable.

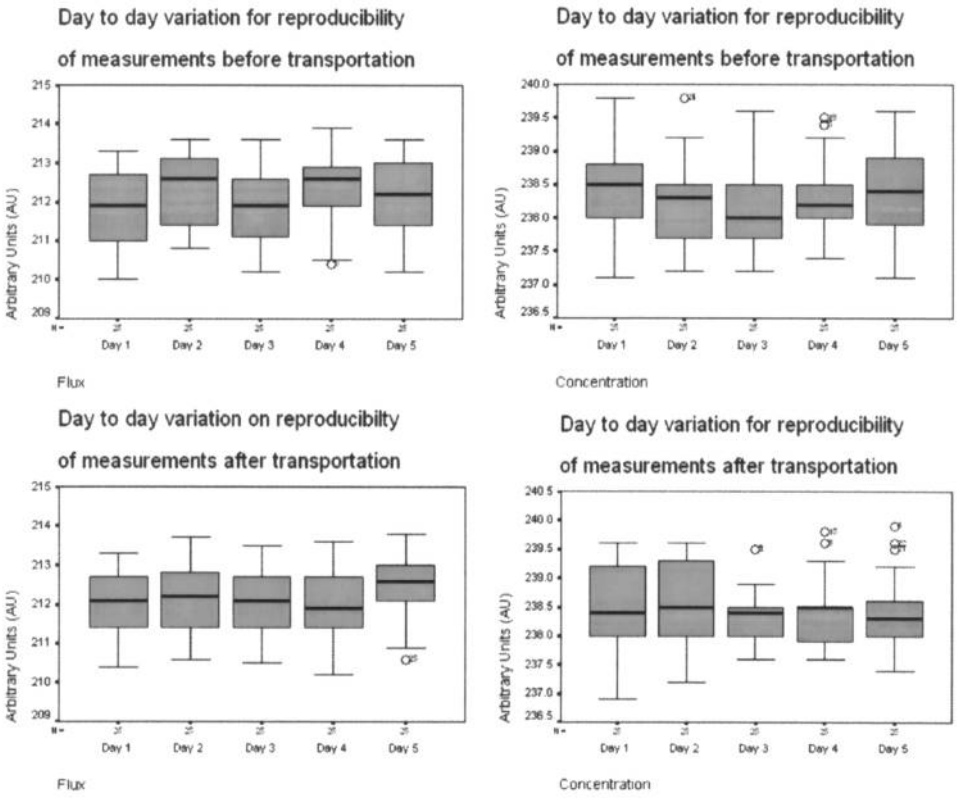


Figure 4-40: Boxplots illustrating the reproducibility of day to day variation laser Doppler fluxmeter measurements for flux and concentration before and after dismantling, transportation and reassembly.

Before or after transport	Flux or concentration	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentiles 5th	Percentiles 95th	90% Range
Before	Flux (Day 1)	211.9	210.0	213.3	211.0	212.9	1.9	210.2	213.3	3.1
Before	Flux (Day 2)	212.6	210.8	213.6	211.4	213.1	1.7	210.8	213.5	2.7
Before	Flux (Day 3)	211.9	210.2	213.6	211.0	212.6	1.6	210.4	213.3	2.9
Before	Flux (Day 4)	212.6	210.4	213.9	211.7	213.0	1.3	210.4	213.9	3.5
Before	Flux (Day 5)	212.2	210.2	213.6	211.4	213.0	1.6	210.4	213.5	3.1
After	Flux (Day 1)	212.1	210.4	213.3	211.4	212.7	1.3	210.5	213.3	2.8
After	Flux (Day 2)	212.2	210.6	213.7	211.4	212.8	1.4	210.7	213.6	2.9
After	Flux (Day 3)	212.1	210.5	213.5	211.4	212.8	1.4	210.5	213.5	3
After	Flux (Day 4)	211.9	210.2	213.6	211.4	212.7	1.3	210.4	213.5	3.1
After	Flux (Day 5)	212.6	210.6	213.8	212.0	213.1	1.1	210.7	213.7	3
Before	Concentration (Day 1)	238.5	237.1	239.8	237.9	238.9	1	237.1	239.7	2.6
Before	Concentration (Day 2)	238.3	237.2	239.8	237.6	238.5	0.9	237.3	239.6	2.3
Before	Concentration (Day 3)	238.0	237.2	239.6	237.7	238.7	1	237.2	239.5	2.3
Before	Concentration (Day 4)	238.2	237.4	239.5	238.0	238.5	0.5	237.6	239.5	1.9
Before	Concentration (Day 5)	238.4	237.1	239.6	237.8	239.0	1.2	237.1	239.6	2.5
After	Concentration (Day 1)	238.4	236.9	239.6	238.0	239.2	1.2	237.2	239.6	2.4
After	Concentration (Day 2)	238.5	237.2	239.6	238.0	239.4	1.4	237.2	239.6	2.4
After	Concentration (Day 3)	238.4	237.6	239.5	238.0	238.5	0.5	237.7	239.5	1.8
After	Concentration (Day 4)	238.5	237.6	239.8	237.9	238.6	0.7	237.6	239.7	2.1
After	Concentration (Day 5)	238.3	237.4	239.9	238.0	238.7	0.7	237.4	239.8	2.4

Table 4-30: Summary of day to day variability on reproducibility of results for test re-tests of flux and concentration measurements of latex spheres in a suspension using the laser Doppler fluxmeter DRT4. A total of 25 repeated measures were taken for the flux and concentration before and after transportation. All results are in arbitrary units.

The boxplots above (see Figure 4-40) illustrate the day-to-day measurement variability for flux and concentration before and after dismantling, transportation and reassembly of the equipment. The x-axis represents the days the measurements were taken and the number of recordings, that is, twenty-five measurements of flux and concentration each day. The y-axis represents the unit of measure used, that is arbitrary units (AU). The two boxplots on the left show flux and the two on the right concentration. The before transportation of equipment are shown on the top boxplots and after in the bottom two. The boxplots indicate a good degree of reproducibility, with tests after transportation showing the greatest consistency. There are some outliers present in all four boxplots that lie within 1.5 times the interquartile range. The medians for flux are close and range from between 211.9 to 212.6 AU. The ranges, percentiles, minimum and maximum values for flux also indicate closeness of agreement (see

Table 4-30). Similarly, the medians for concentration indicate closeness with a range of 238.0 to 238.5 AU. The minimum and maximum values, ranges and percentiles also show a high degree of closeness. Overall, the above boxplots and tables indicate closeness of agreement and consistency of test re-test measurements taken over five different days for before and after relocation of the laser Doppler fluxmeter unit.

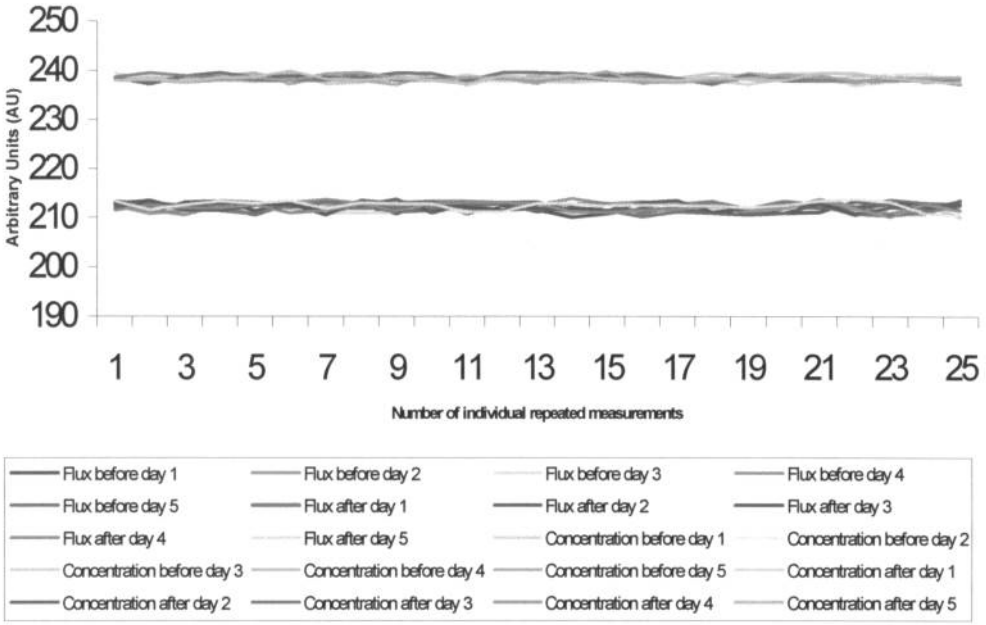


Figure 4-41: Line graph showing day to day variation for the individual 25 repeated measurements of flux and concentration for before and after dismantling, transportation and reassembly of the laser Doppler fluxmeter unit.

A line graph was constructed (see Figure 4-41) to investigate the reproducibility of individual measurements taken for flux and concentration over five different days. The x-axis represents the number of individual repeated measurements taken, that is, 25 test re-test measurements each day. The y-axis represents the arbitrary units of measurements for the laser Doppler fluxmeter values. Overall, the line graph indicates consistency in reproducibility of repeated measurements taken over a period of five days for before and after.

No of measurements	Measurement	Variable	Median	Minimum	Maximum	Percentiles		IQR	Percentiles		90% range
						25th	75th		5th	95th	
25	Day 1	Flux	212	210	213	211	213	2	210	213	3
25	Day 2	Flux	213	211	214	211	213	2	211	214	3
25	Day 3	Flux	212	210	214	211	213	2	210	213	2
25	Day 4	Flux	213	210	214	212	213	1	211	214	3
25	Day 5	Flux	212	210	214	211	213	2	210	214	3
25	Day 1	Conc.	239	237	240	238	239	1	237	240	3
25	Day 2	Conc.	238	237	240	238	239	1	237	240	2
25	Day 3	Conc.	238	237	240	238	239	1	237	240	2
25	Day 4	Conc.	238	237	240	238	239	1	238	239	2
25	Day 5	Conc.	238	237	240	238	239	1	237	240	2

Table 4-31: The analysis of within measurements for the day-to-day variability before dismantling, transportation and reassembly of the equipment. The analysis relates to 25 repeated measurements carried out over 4 different days. All data is in perfusion Arbitrary Units.

To study the within measurement variability for the measurements taken on 5 different days the median, minimum and maximum values, the 5th, 25th, 75th and 95th percentiles and the interquartile and 90% ranges were investigated. From Table 4-31 the closeness of agreement between the 25 repeated measurements, for the parameters described in the table, taken on different days indicates reproducibility of the within measurements taken on each day for before dismantling the equipment. Similarly, Table 4-32 also indicates closeness of agreement for the within 25 repeated measurements taken on 5 different days for after the reassembly of the measuring equipment.

No of measurements	Measurement	Variable	Median	Minimum	Maximum	Percentiles		IQR	Percentiles		90% range
						25th	75th		5th	95th	
25	Day 1	Flux	212	210	213	211	213	1	211	213	2
25	Day 2	Flux	212	211	214	211	213	1	211	213	3
25	Day 3	Flux	212	211	214	211	213	1	211	213	3
25	Day 4	Flux	212	210	214	211	213	1	211	213	2
25	Day 5	Flux	213	211	214	212	213	1	211	213	2
25	Day 1	Conc.	239	237	240	238	239	1	237	239	2
25	Day 2	Conc.	239	237	240	238	239	1	237	239	2
25	Day 3	Conc.	239	237	240	238	239	1	237	240	2
25	Day 4	Conc.	238	238	240	238	239	1	238	239	1
25	Day 5	Conc.	239	238	240	238	239	1	238	240	2

Table 4-32: The analysis of within measurements for the day-to-day variability after dismantling, transportation and reassembly of the equipment. The analysis relates to 25 repeated measurements carried out over 4 different days. All data is in perfusion Arbitrary Units.

4.4.2.6 Study 13: Range, limit of detection and limit of quantification of laser Doppler fluxmeter

To test the dynamic range of the laser Doppler fluxmeter an *in vivo* study was chosen. Sixteen healthy volunteers took part in the study. Room temperature was kept within the range of 21.6 ±0.9°C. The general exclusion criteria and measures to reduce experimental errors described in Appendix 1 were followed. A foot was selected at random. The dynamic range of the laser Doppler fluxmeter was measured by applying plantar foot pressure to the point were biological zero was reached for a period of 3 minutes with the subject in a semi-weight bearing position. Following release of arterial arrest the hyperaemic response was measured. The procedure was repeated for all subjects. The minimum range of the scale was taken as the lowest biological zero value. The maximum hyperaemic response was taken as the maximum tested range for the measuring instrument.

The limit of detection was calculated as the lowest value that was detected in the range study nearest to the biological zero. The limit of quantification was determined as the lowest value that was detected by the laser Doppler

fluxmeter in the *in vitro* pig's blood flow model (see section 4.4.2.3, page 148) as controlling and quantifying skin blood flow *in vivo* is difficult.

	Minimum	Maximum	Range
Flux	2.3	929.5	927.2
Concentration	2.6	1000.0	997.4
Speed	0.9	867.2	866.3

Table 4-33: The *in vivo* minimum and maximum values and range for the laser Doppler fluxmeter DRT4. All values are in arbitrary units (AU)

The *in vivo* tested range (see Table 4-33) for blood cell flux is 927.2 AU with minimum and maximum values of 2.3 and 929.5 AU respectively. For the concentration of blood cells the tested range is 997.4 AU with a minimum value of 2.6 and maximum value of 1000.0 AU. Finally, the minimum blood cell speed is 0.9 AU and a maximum of 867.2 AU with a range of 866.3 AU. From the *in vitro* pig's blood flow model the limit of quantification was calculated as 0 AU for flux, 54.8 AU for concentration of blood cells and 0 AU for blood cell speed.

4.5 Discussion

Linearity between two measuring systems shows that the output of one measuring system is directly proportional to the output of the second measuring system, that is, they are correlated along a straight line (Field, 2002; Bolton, 2000; Kinnear *et al*, 2000). The strain gauge output showed an excellent linear correlation against applied pressure although the system used to convert voltage output to pressure using a regression equation might have introduced a small systemic error. The laser Doppler fluxmeter has been correlated with other validated systems to measure skin blood flow with excellent results (Johnson, 1990; Almond *et al.,* 1988; Oberg *et al.,* 1983; Holloway *et al.,* 1977). One of the validation studies correlated the laser Doppler fluxmeter with an *in vitro* pig's blood flow model. This model was chosen for the *in vitro* linearity experiment because of similarities between human blood and porcine blood (Weng *et al.,* 1996; Reifenberg *et al.,* 1989; Chi-Chung *et al.,* 1985). The model demonstrated a fair correlation, although there were some limitations. The model measured

blood flow by calculating the amount of blood fluid displaced over a given amount of time. The laser Doppler fluxmeter measures blood flow relative to the speed and number of mainly red blood cells that passed by the measuring probe. When the burette was filled with blood the red blood cells immediately commenced to settle, increasing the concentration of cells near the tap over the five seconds before the tap was open. However although limitations existed, especially since the two systems were measuring different variables of blood, a fair level of linearity was found between the measured model's blood volume speed and laser Doppler fluxmeter blood cell speed. Other studies have found a close correlation between the laser Doppler fluxmeter and other equipment used to measure skin blood flow (see Table 4-34) suggesting that the laser Doppler fluxmeter produces a fair to excellent correlation between measured value and skin blood flow depending on the model/equipment used. From Table 4-34 the laser Doppler fluxmeter produced the highest correlation with the laser Doppler imager because both systems measure skin blood flow using the same principle. The other systems measure skin blood flow using other methods like the rate of absorption of a radioisotope by the microcirculation for ^{133}xenon clearance and the rate of disappearance of hydrogen from perfused tissues by hydrogen clearance technique. The fact that a fair linearity was found between the *in vitro* porcine blood flow model and the laser Doppler fluxmeter was more due to the limitations of the validation model used rather than the skin blood flow measurements carried out by the laser Doppler fluxmeter. Thus within the parameters measured of measuring blood flow relative to the Doppler effect of moving blood cells the laser Doppler fluxmeter appears to produce excellent linearity with similar systems and suggests that publications measuring skin blood flow using different equipment is not directly comparable since the system used to calculate blood flow and the units of measure are different.

Authors	Equipment compared against LDF	r	r^2	P value
Holloway *et al.* (1977)	^{133}xenon clearance blood flow	0.89	0.79	P<0.001
Seifalian *et al.* (1994)	Laser Doppler imager	0.96	0.92	P<0.01
Kvietys *et al.* (1985)	Hydrogen clearance technique	0.73	0.53	P<0.0001

Table 4-34: Comparison between the laser Doppler fluxmeter and other systems are shown. From the published studies using the r values the r^2 values were calculated.

The accuracy of a measuring system is worked out by testing the system over a given range a number of times, followed by calculating the average error, which can also be described as a percentage of the full-scale deflection of the measuring instrument. The results of the validation studies indicate that the accuracy of the pressure and skin blood flow systems is within acceptable levels for the intended use of the equipment.

The sources of error that can affect the results obtained using any measuring system is classified into random errors and systematic errors. Random errors comprise operating errors (for example, inter or intra-tester errors, subject variability), environmental errors (for example, room temperature and humidity, temperature of equipment) and stochastic errors, that is, noise (for example, unwanted signals that are picked up by the system and interfere with the signal being measured). Systematic errors relate to construction errors (for example, errors that may occur during the manufacturing of the system as a result of tolerances occurring during the construction of equipment, materials and electrical components used), approximation errors, that is, accepted assumptions regarding the relationships between two quantities (for example, during calibration a linear relationship between the standard and measuring system's output value is assumed but in practice it is only an approximation to the true relationship), ageing errors (for example, wear and tear on measuring system's components affecting output values) and insertion errors, that is, inserting the instrument into a position or casing that may affect the quality of its value (Bolton, 2000). Although the manufacturers' of the strain gauge and laser Doppler fluxmeter systems provided equipment validation results for their respective systems, due to their use in a new application (that is, to measure the effects of plantar foot pressure on skin blood flow) the

manufacturers' results may not be representative of the results obtained under the study's operating conditions, hence both sets of equipment were extensively validated under the required operating conditions and with the transducers embedded in the shoe device.

The sources of error that may have led to the reported levels of linearity, accuracy, repeatability, and reproducibility for the integrated skin blood flow and plantar foot pressure system are multi-factorial. With regards to operating errors using the strain gauge pressure measuring system, the system was designed for single operator use hence inter-tester operator error would not affect the accuracy of the pressure system and since the display unit was digital the parallax errors that occur in reading the position of the pointer on the scale in such systems cannot occur when measuring pressure alone. Similarly, operator errors would not affect the skin blood flow system since a single operator used the system. However, when using the integrated skin blood flow and plantar foot pressure measuring system the fact that the system was visually/sound synchronised suggests that an operator error could be introduced when data recording. In addition, the results for the integrated system can also be affected by operating errors due to inter-subject variation.

To reduce environmental errors affecting the results a number of measures were taken to reduce the number of environmental variables that could affect the integrated measuring system (see Appendix 1). To achieve this room temperature and humidity was controlled during the calibration process and during all the validation studies conducted. In addition, since the laser Doppler fluxmeter may be affected by environmental background vibration no data recording was carried out when heavy machinery was in operation in proximity to the experimental room. Furthermore, extreme environmental bright light could also affect skin blood flow measurements so all experiments were conducted with the lights in the room dimmed and away from windows to prevent any sunlight affecting recordings. Another factor that could produce a random error and affect results is the equipment temperature, specifically the changes in temperature that occurs as

electronic equipment warms up from cold. Thus, the integrated system was tested for the effects of warm-up from cold over a three-hour period. The resistance of strain gauges are not only known to be affected by a change in applied strain but also by a change in the temperature of the strain gauge itself (Bolton, 2000). The strain gauge is a length of wire or metal foil or semi-conductor and works on the principle that when it is stretched or compressed its resistance changes. Most strain gauges operate on the principle that when they are compressed resistance decreases and when they are put under tension the resistance decreases (Bolton, 2000). Thus, heat would affect the material properties and its resistance. The validation experiments revealed that the strain gauge appears to be minimally affected by equipment warm-up within the tested range. The laser Doppler fluxmeter was also tested and the validation studies suggested that equipment warm-up did not significantly affect the measurements however it was recommended that the system should be warmed-up for 1-hour prior to use since data recorded after this period of time appeared to stabilise.

It was detected that mobile phones produced a stochastic error when operated near the integrated system hence all subjects were asked to switch off their mobile phones before entering the experimental room. In addition all connections were checked for cleanliness and a procedure for checking all the connections within the integrated system, every time the system was assembled, was introduced to reduce any noise resulting from poor connections. These experimental precautions taken assisted in reducing stochastic errors and would have contributed to the overall non-significant differences found between before and after dismantling, transportation and reassembly of the experimental equipment.

When constructing the three-tier piston and the shoe device all possible construction errors were minimised. All the tolerances for the construction of the three-tier piston and its mechanism were kept to the nearest millimetre of the design. Such precision in the construction of the remainder of the shoe device was not deemed necessary and the tolerances were higher. Thus construction errors were reduced to minimum possible.

One of the main sources of error affecting the accuracy, repeatability and reproducibility of the developed system was approximation error. In order to convert the voltage output value into pressure a number of approximation errors were introduced. In order to carry regression it was assumed that good correlation existed between the voltage output of the strain gauge and applied force/pressure. Although the results show excellent correlation between both variables the correlation is not 100% perfect thus the regression produced an excellent regression but not a perfect one. The regression and other formulae were then used to convert voltage output to pressure using the "strain gauge conversion calculator" (see Appendix 3). Thus, such system is also liable to the mathematical computation errors of the computer system used. Finally, the calibration method was more indicative of the application of force rather than pressure although equations were used to convert to pressure.

Ageing errors are unlikely to have affected the system during the studies, as wear and tear of materials would not have occurred during the comparatively small duration of time that the equipment was used. Both the strain gauge and the laser Doppler fluxmeter systems have been purchased recently and new probes were fitted to the DRT4. Finally, encasing the pressure and skin blood flow transducers within a three-tier piston and within the shoe and its spindle mechanism to apply or remove pressure may have led to the introduction of insertion errors. Thus, it was important, were possible to test and validate the system within its operating environment to account for this type of error.

Precision describes the closeness of agreement or the degree of scatter that occurs as a result of random errors between a number of measurements taken from multiple recording sessions of the same homogenous group under controlled conditions (Bolton, 2000; Bircher *et al.,* 1994). The precision of a measuring instrument describes the instrument's repeatability and reproducibility (Bircher *et al.,* 1994).

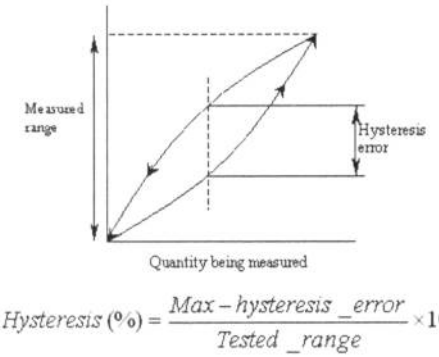
The repeatability is the ability of a measuring instrument to record the same value for repeated measures under the same experimental conditions, that

is, repeated measures of the same value of the quantity being measured (identical sample) are taken by the same operant using the same equipment and the same conditions (Bolton, 2000; Bircher *et al.,* 1994). The strain gauge showed a high level of consistency and repeatability both for grouped and individual analysis of the data presented. The laser Doppler fluxmeter also showed a high level of repeatability for the *in vitro* studies, both for grouped and individual analysis of the data, where variables were easier to control. For the *in vivo* validation studies a greater degree of variability was found, due to the difficulty in controlling intra-individual measurement site variability and the complex nature in controlling vascular responses which involve both local and central mechanisms. As a result a number of authors have reported a high variation for repeated measurements (see Chapter 6, Section 6.7 Discussion). Simply turning the probe at 90 degrees is sufficient to affect repeatability results. Gush *et al.* (1984) reported 10% variability for measurements taken on the same site and greater than 10% for repeated measurements taken between adjacent sites. Considering that the foot was taken in and out of the measurement shoe for each repeated measure the fact that the values for the coefficient of variation are close to 10% suggests the equipment is operating correctly *in vivo* and the variation in repeated measurements are close to that reported by other authors. However, more validation of the laser Doppler fluxmeter should be done *in vivo,* as it would be more representative of its true operating conditions.

The reproducibility of a measuring instrument describes the ability of the instrument to produce consistent results under different conditions, that is, on different days, during equipment warm-up, when using different laser Doppler fluxmeters or different operators (Bolton, 2000; Bircher *et al.,* 1994; Fowkes *et al.,* 1991). Fowkes *et al.* (1991) states that many researchers pay too little attention to the reproducibility of measurements and that such evidence should be sought in the published article. Some researchers refer to reproducibility as the reliability of the measuring instrument (Wood-Dauphinee *et al.,* 1989). Two reproducibility validation studies were carried out for the strain gauge and the laser Doppler fluxmeter, one tested the

effects of equipment warm-up (that is, repeated measures under different equipment temperatures) and the second the effects of day-to-day variation. Both the strain gauge and the laser Doppler fluxmeter showed overall consistency and reproducibility of test re-test during equipment warm-up and measurements taken on different days within acceptable study standards.

Some types of equipment, especially pressure measurement systems, may be affected by hysteresis. Some electrical and mechanical instruments when measuring known standard values produce different readings depending on whether the measurements are taken by increasing or decreasing change (Bolton, 2000; Currier, 1990). In systems with a mechanical application hysteresis is the result of energy being absorbed by the system during loading and not released during unloading (Currier, 1990). Thus, for example, the materials used to construct a strain gauge may absorb energy during loading but not release this retained energy quick enough during unloading, hence the differences in the resistance of the strain gauge would produce different results for loading and unloading for the same applied load. Hysteresis is calculated by dividing the maximum hysteresis error by the range tested and multiplied by 100 (see Figure 4-42). The strain gauge tested produced an acceptable hysteresis of 0.73% for the range 0 to 100 kPa.



$$Hysteresis\ (\%) = \frac{Max - hysteresis\_error}{Tested\_range} \times 100$$

Figure 4-42: Method of calculating the hysteresis is shown above (Bolton, 2000).

The range of an instrument describes the limits between which measurements can be taken, where the instrument has been proven with regards to accuracy, precision and linearity (Bolton, 2000; Bircher *et al.*,

1994). Since the measuring system may saturate and may miss important changes outside the validated range, it is vital that any study results are within the validated range for the equipment (that is, where the accuracy, precision and linearity of the system within those limits is known) (West *et al.,* 1999). Following the validation studies the pressure system may be used for the measurement of plantar foot pressure from 0 to 100 kPa. The range for the laser Doppler fluxmeter was determined by calculating the biological zero (minimum value) and the post-occlusive hyperaemic response (maximum value) (Bircher *et al*, 1994; Wahlberg *et al.,* 1992; Fagrell, 1990). The *in vivo* range for the laser Doppler fluxmeter is 2.3 to 929.5 AU for flux, 2.6 to 1000.0 AU for concentration of blood cells and 0.9 to 867.2 AU for speed of blood cells.

The sampling frequency of a system is the number of times a transducer is scanned over a given period of time. Caution should be taken with some systems as the manufacturers quote a high sampling frequency but in fact the frequency for which the entire matrix of transducers is scanned and the true sampling frequency is much lower because it is the number of times each individual transducer is scanned (West *et al.,* 1999). A high sampling is necessary for dynamic activity since a slow system will miss specific events. Since the device to measure the effects of plantar foot pressure on skin blood flow is not a dynamic system a high sampling frequency is not needed however, the sampling frequency for the pressure system and the laser Doppler fluxmeter was 40 Hertz for both and allowed for synchronisation of data for analysis.

The spatial resolution of a measuring instrument is the number of transducers in one square centimetre (West *et al.,* 1999). The spatial resolution required for a study is dependant on the structure to be measured and the type of study. To measure plantar foot pressure the higher the spatial resolution the clearer the pressure map for the foot, the lesser the risk of missing small areas and the lesser the risk of under estimating pressure (West *et al.,* 1999). However since the device to measure the effects of plantar foot pressure on skin blood flow only needs to measure

the pressure over the area sampled by the laser Doppler fluxmeter, only one pressure transducer of the same size as the laser Doppler fluxmeter transducer's sampling area was used.

The experimental device was developed so that it was portable and could be dismantled, transported and reassembled in different locations where the data collection was to take place. Thus, the validation studies investigated the effects of this on accuracy, repeatability and reproducibility of results. This was termed "ruggedness" of the device. The validation studies concluded that the experimental measuring system was not significantly affected by moving equipment to a new place and is suitable for use in different locations.

4.6 Conclusion

This chapter explains the development and validation of an experimental system for measuring the effects of plantar foot pressure on skin blood flow. The experimental system comprises of a strain gauge to quantify plantar foot pressure, which is applied and removed using a spindle mechanism to the centre of the heel. Skin blood flow is measured using the laser Doppler fluxmeter system. By integrating the strain gauge and the laser Doppler fluxmeter transducers into a three-tier piston system both variables acting on the same area of the skin was quantified. The validation studies indicate that the experimental system is suitable for measuring the effects of plantar foot pressure on skin blood flow under the intended conditions. The system can be used to record both in the supine and semi-weight bearing positions.

4.7 Limitations and recommendations

Human walking involves an upright posture and the postural venoarteriolar response physiologically regulates blood flow when persons move from a lying position to a sitting or standing position. However, little is known as to the effects of plantar foot pressure on skin blood flow when in the supine and an upright or semi-weight bearing position. A study should be carried out with the aim of investigating how the postural venoarteriolar response affects the effects of plantar foot pressure on skin blood flow. Since the purpose of this thesis is to assess the effects of plantar foot pressure on

skin blood flow in patients with rheumatoid arthritis and plantar foot pressure occurs when the patient stands or walks, if differences do exist between both positions (that is, supine and semi-weight bearing) then this thesis should concentrate on the semi-weight bearing effects.

An important limitation of the developed system is that the plantar foot pressure and skin blood flow systems were visually/sound synchronised and a system to electronically synchronise both systems should be developed.

Since the errors between the *in vivo* and *in vitro* studies vary for the laser Doppler fluxmeter, more *in vivo* validation should be carried out, as this is more representative of the true operating environment of the studies described in later chapters.

The piston surface area should be reduced to the size of the laser Doppler fluxmeter DP1T/7-4 transducer so that the applied pressure is applied more precisely to the area where skin blood flow is being measured.

CHAPTER 5

AN INVESTIGATION INTO THE EFFECTS OF POSTURAL CONTROL ON PLANTAR FOOT PRESSURE AND SKIN BLOOD FLOW

5. CHAPTER 5: An investigation into the effects of postural control on plantar foot pressure and skin blood flow

5.1 Introduction

Change in posture is known to have an effect in vascular haemodynamics due to the venoarteriolar response. Findings in Chapter 4 indicated a reduction in skin blood flow following a change in posture from supine to upright sitting and similar findings have been reported. Prior to developing a study protocol to investigate the effects of plantar foot pressure on skin blood flow in subjects with rheumatoid arthritis it is important to carry out a preliminary investigation to establish whether applying the same amount of plantar foot pressure on the centre of the heel in the supine and semi-weight bearing positions produces different results. That is, is the percentage reduction in plantar foot pressure during the application of quantifiable plantar foot pressure dependant on postural position? Similarly, is the hyperaemic response between the two positions different? Since the futures studies relate to plantar foot pressure and human walking involves an upright stance it is important to answer the above questions, as this will influence the rheumatoid arthritis study protocol. Should differences be present then the future protocol should adopt an upright posture for measuring the effects of plantar foot pressure on skin blood flow as this would be more indicative of the physiological effects of ambulation on the tissues on the plantar aspect of the foot.

Since posture has an effect on blood flow to the lower limbs, with vasoconstriction occurring when humans move from a supine to a standing position, the venoarteriolar response therefore appears to be an evolutionary measure that has an important function in maintaining physiological homeostasis relative to posture and gravity. Its complete function has not yet been fully investigated although there are established theories with regards to its role in preventing oedema. Following the recommendations in Chapter 4 (see section 4.7, page 176), this study was carried out with the aim of investigating the relationship between the

postural venoarteriolar response and the effects of plantar foot pressure on skin blood flow. The results of this study will be used to determine which position subjects will adopt in the rheumatoid arthritis study investigating the effects of plantar foot pressure on skin blood flow.

5.2 Method

5.2.1 Subjects

Ethical approval was obtained from the ethics committee at Queen Margaret University College. A total of four volunteers (two males and two females) participated in the study with an age distribution of 28 ±8.6 years. See Appendix 1 for subject exclusion criteria and measures taken to reduce variables that may affect skin blood flow. The evidence for taking these measures to reduce variables affecting skin blood flow has been discussed in Chapter 4 (section 4.5). Room temperature and humidity were kept at 21.7 ± 0.8°C and 40 ± 3.4% respectively.

Subjects were randomised into a supine position followed by a semi-weight bearing position or a semi-weight bearing position followed by a supine position. Subjects acclimatised in the randomly assigned starting position.

5.2.2 Recording procedure

To achieve a reproducible system the foot was placed in the shoe ensuring the heel was in contact with the heel counter and the midline of the 3rd digit was aligned with a mark on the shoe prior to fixing the foot with the shoe straps.

5.5.2.1 Supine recording procedure

The shoe device was placed in the supine position (see Figure 4-7, page 109). The randomised right or left foot was then placed in the shoe at zero piston pressure, skin temperature was checked and a baseline recording taken for 2 minutes. One of the randomised plantar foot pressure values was then applied, by turning the spindle, and cutaneous blood flow recorded for 3 minutes. After the pressure was removed cutaneous blood flow was recorded for 5 minutes as the signal returned to reference baseline. After an additional 5-minute recovery period the procedure was repeated for the

other two pressures. The plantar foot pressure values used were 20 kPa, 40 kPa or 80 kPa and were randomised for each subject. These pressures were used because these were some of the pressures used by Meinders *et al.* (1996) in a study investigating the effects of plantar foot pressure on skin blood flow in the heel of healthy subjects. Using these pressures would allow comparisons to be made of findings in the supine position between studies.

5.5.2.2 Semi-weight bearing recording procedure

The shoe device was placed in the semi-weight bearing frame (see Figure 4-7, page 109) to allow for semi-weight bearing measurements to be taken. The recording procedure described above was repeated in this new position.

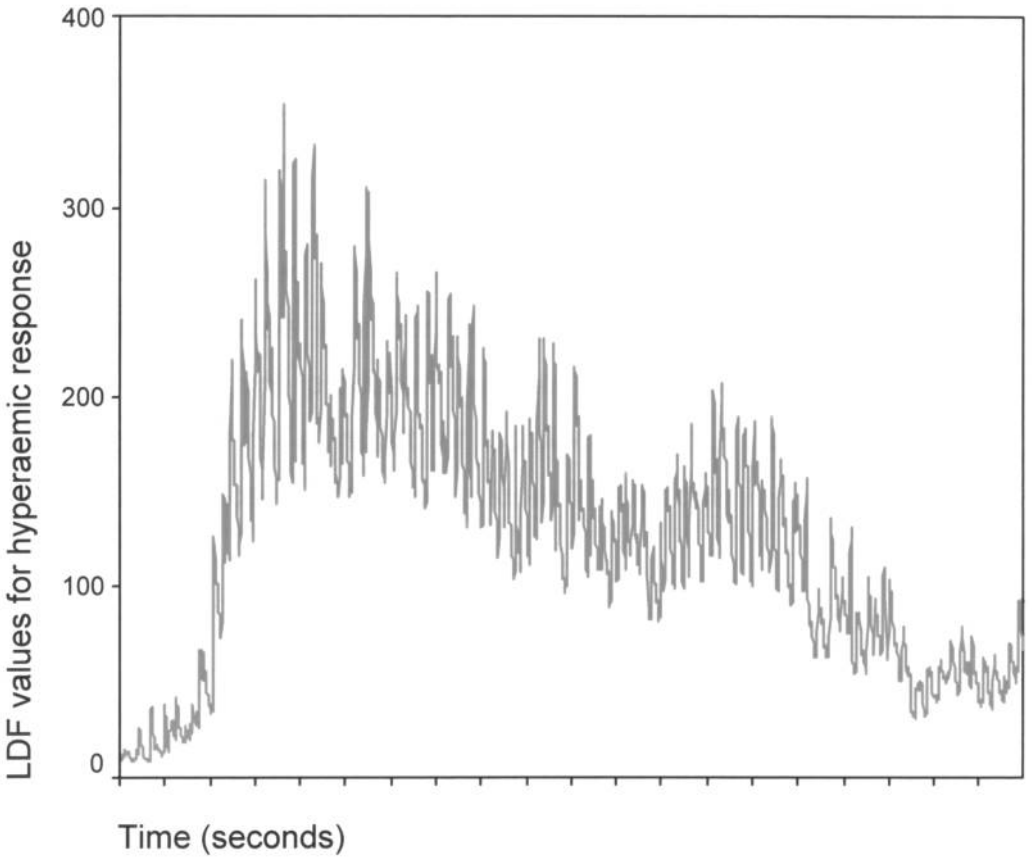


Figure 5-1: An example of the hyperaemic response of a laser Doppler fluxmeter flux following the release of 3-minutes of pressure on the plantar aspect of the heel.

5.3 Results

5.3.1 Data analysis

Data analysis compared supine against semi-weight bearing and was divided into two parts: response to loading and the hyperaemic response post-release of pressure. The hyperaemic response post-pressure application may suggest a registration of insult to the soft tissues (see Figure 5-1). The x-axis represents the time in seconds and the y-axis the flux values in perfusion Arbitrary Units. The trace does not show the effects of applying pressure from baseline but the effects of the release of pressure. Upon the release of pressure a hyperaemic response occurs which settles to baseline after a few minutes. Other studies have reported findings by interpreting the hyperaemic response (Meinders *et al.,* 1996; Bircher *et al.,* 1994; Netten *et al.,* 1993; Kristensen *et al.,* 1986). All laser Doppler flowmetry readings are in perfusion arbitrary units (AU). The baseline skin blood flow was recorded for 2 minutes prior to the application of pressure in the supine and semi-weight bearing positions. A typical example of a laser Doppler fluxmeter signal output for the registration of a baseline recording on the plantar aspect of the heel is shown below (see Figure 5-2) for the unloaded period prior to the application of pressure. The x-axis represents the time in seconds and the y-axis the flux taken at baseline. The flux signal produced by the laser Doppler fluxmeter is constantly fluctuating as a result of cardiac rhythm, the speed and concentration of blood cells, and other random fluctuations.

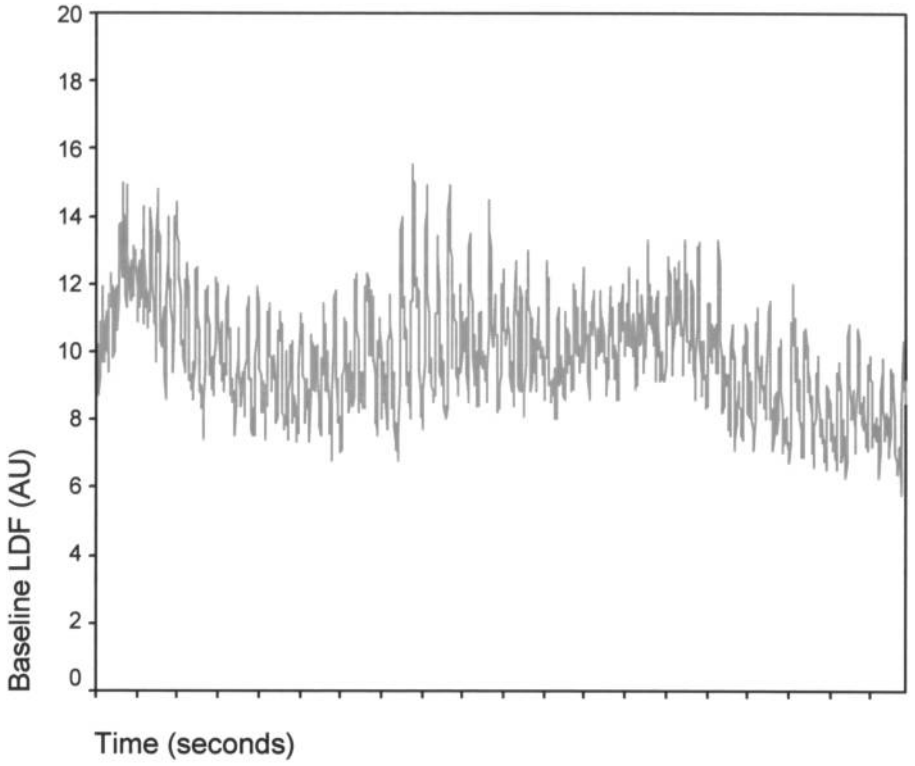


Figure 5-2: An example of a baseline laser Doppler fluxmeter signal on the plantar aspect of the heel for flux.

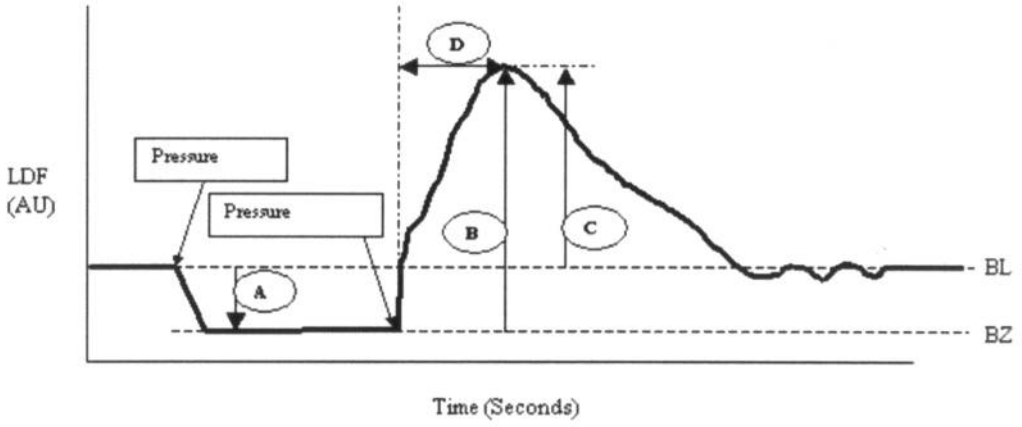


Figure 5-3: The analysis of the laser Doppler fluxmeter curve is described. The laser Doppler flux values are in perfusion in Arbitrary Units (AU); BL refers to baseline laser Doppler fluxmeter measurement; BZ refers to biological zero; A is the decrease in laser Doppler fluxmeter flux following the application of pressure; B is the maximum peak laser Doppler fluxmeter flux attained from BZ following release of pressure; C is the absolute peak laser Doppler fluxmeter flux attained from BL following release of pressure; and D is the time taken in seconds to reach the highest laser Doppler fluxmeter flux.

All data was referenced to biological zero, with biological zero being the residual noise offset of laser Doppler fluxmeter measurements in the absence of blood and is higher than instrument zero (Bircher *et al.*, 1994).

5.5.3 Laser Doppler fluxmeter levels during pressure loading

The laser Doppler fluxmeter level during the application of plantar foot pressure (A in Figure 5-3) is the decrease in flux following the application of external pressure for a total period of three minutes.

5.5.4 Hyperaemic response

The hyperaemic response was divided into three parts:

Maximum peak hyperaemic response (B in Figure 5-3) – the maximum rise in flux following release of plantar foot pressure.

Peak hyperaemic response relative to baseline (C in Figure 5-3) – the maximum rise in flux following the release of plantar foot pressure from the baseline reading.

Time to peak hyperaemic response (D in Figure 5-3) – time taken from the release of plantar foot pressure to reach the highest flux value of the hyperaemic response.

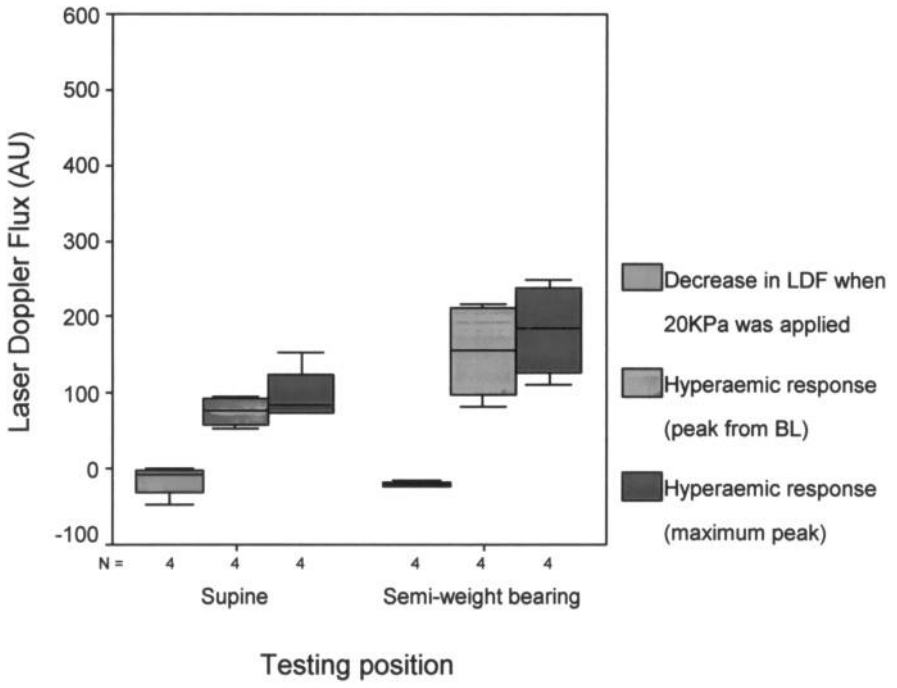


Figure 5-4: The effects of applying 20KPa of pressure in two positions to the skin for 3 minutes and the resultant hyperaemic response. LDF refers to laser Doppler flux and BL to baseline.

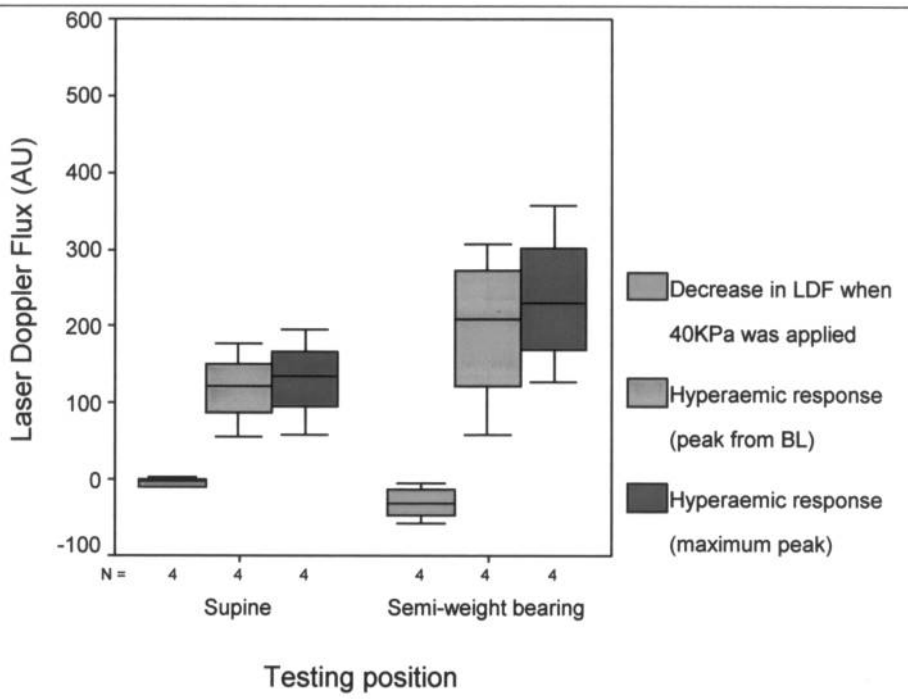


Figure 5-5: The effects of applying 40KPa of pressure in two positions to the skin for 3 minutes and the resultant hyperaemic response. LDF refers to laser Doppler flux and BL to baseline.

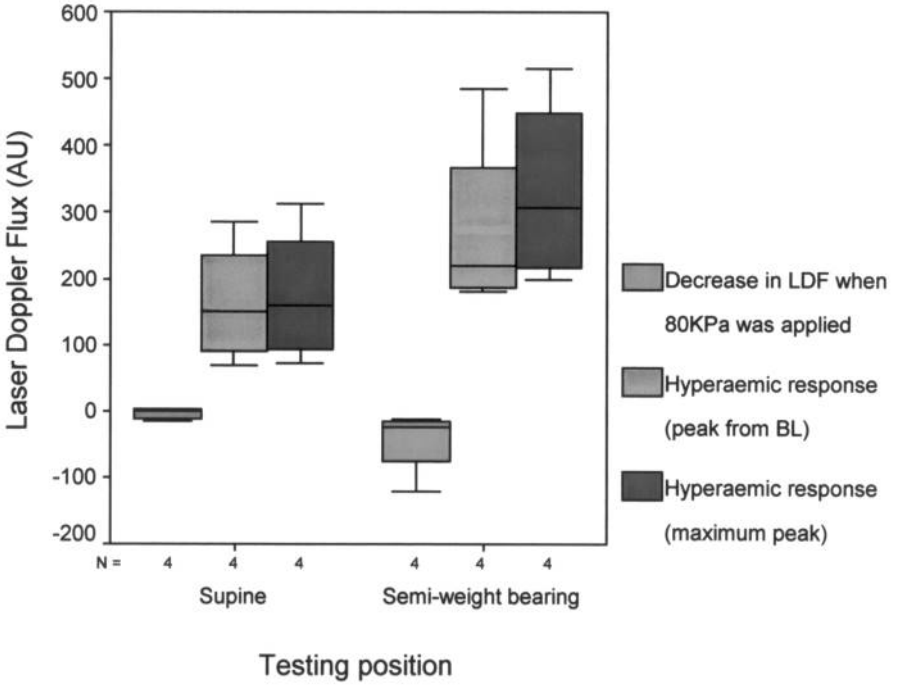


Figure 5-6: The effects of applying 80KPa of pressure in two positions to the skin for 3 minutes and the resultant hyperaemic response. LDF refers to laser Doppler flux and BL to baseline.

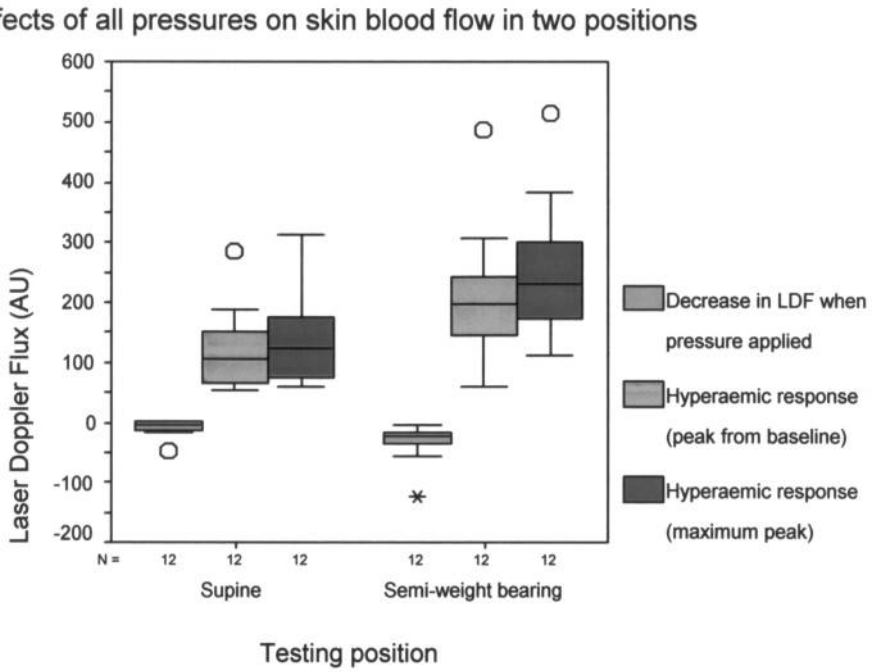


Figure 5-7: The effects of applying all the various pressures in two positions to the skin for 3 minutes and the resultant hyperaemic response. LDF refers to laser Doppler flux and BL to baseline.

5.5.5 Laser Doppler fluxmeter levels during pressure loading

The boxplots above show the effects of the application of plantar foot pressure to skin blood flow measured as laser Doppler fluxmeter flux. The x-axis in Figure 5-4 to Figure 5-7 represents the measurement position, which is supine or semi-weight bearing. At each position the decrease in laser Doppler flux when plantar foot pressure was applied, the hyperaemic response from baseline and the maximum hyperaemic response are shown on each boxplot. The y-axis represents the measured laser Doppler flux in perfusion arbitrary units. Figure 5-4 shows the effects of applying 20 kPa of pressure on skin blood flow, Figure 5-5 and Figure 5-6 the effects of applying 40 kPa and 80 kPa respectively. There are no outliers in the boxplots for the application of 20, 40 or 80 kPa of plantar foot pressure. Figure 5-7 shows the total effect of applying plantar foot pressures of 20, 40 and 80 kPa on skin blood flow. During pressure loading there was a reduction in all the laser Doppler fluxmeter flux values. The reduction in the total laser Doppler flux levels following the application of plantar foot pressure, as shown in Figure 5-7 and Table 5-1, was greater in the semi-weight bearing position when compared to the supine position. However, there are three outliers 1.5 box lengths above and one below the boxes and

one extreme outlier three box lengths below the box for the decrease in laser Doppler flux when plantar foot pressure was applied.

The data was non-parametric due to the small sample size hence a Wilcoxon's paired sample test was carried out to test the hypothesis whether the two related variables (that is, the measured positions) had the same distribution. The Wilcoxon is a non-parametric test and thus makes no assumptions with regards to the shapes of the distribution of the two variables. The test only takes into account the magnitude of the differences within the pairs and gives greater weight to the pairs that show smaller differences. The Wilcoxon's paired sample test showed a significant difference between blood flow reductions following the application of pressure ($p = 0.015$) in the supine (median –4.9 AU) and semi-weight bearing (median –22.4 AU) positions (see Table 5-1). There was no significant increase or decrease in probe/skin temperature between baseline and pressure applied for both positions.

Applied pressure	***Position***	***Pressure applied decrease in flux from baseline***	***Hyperaemic Response***		
			Time to peak	***Max peak***	***Peak from BL***
20 (KPa)	Supine	-8.8 (47.2)	11.9 (64.8)	85.5 (79.1)	77.4 (42.1)
	Semi-weight bearing	-20.6 (6.3)	42.5 (126.4)	185.9 (137.7)	157.0 (134.8)
40 (KPa)	Supine	-3.6 (13.7)	5.2 (195.6)	133.9 (137.7)	121.4 (121.5)
	Semi-weight bearing	-31.1 (52.1)	61.8 (3.7)	230.6 (227.6)	209.1 (247.4)
80 (KPa)	Supine	-2.1 (19.8)	5.0 (36.6)	158.7 (241.7)	149.6 (216.5)
	Semi-weight bearing	-25.1 (109.9)	60.1 (59.2)	307.5 (314.4)	221.1 (306.3)
All pressures	Supine	-4.9 (50.3)	5.2 (197.5)	124.2 (253.9)	104.2 (231.3)
	Semi-weight bearing	-22.4 (117.1)	57.7 (137.4)	230.2 (230.2)	197.35 (426.9)
Wilcoxon's paired samples test between supine and semi-weight positions		P = 0.015	P = 0.034	P = 0.006	P = 0.028

Table 5-1: The median values (range) for the application of various levels of pressure in the supine and semi-weight bearing positions. All laser Doppler flux skin blood flow values are in arbitrary units, except for the "Time to peak" which is in seconds.

Although the Wilcoxon's paired samples test indicated a statistical significant difference between measurements taken in the supine and semi-weight bearing positions the data was non-parametric and the ranges high. Hence, further analysis was carried out to investigate the 5th, 25th, 75th and 95th percentiles and the 90% and interquartile ranges (see Table 5-2 to Table 5-5).

Applied Pressure	Position	Median	Percentiles		IQR	Percentiles		90% range
			25th	75th		5th	95th	
20 kPa	Supine	-8.8	-39.1	-0.8	-38.3	-47.3	-0.1	-47.2
20 kPa	Semi-weight bearing	-20.6	-22.7	-17.3	-5.4	-22.7	-17.3	-5.4
40 kPa	Supine	-3.6	-10.5	-2.0	-8.5	-11.3	-2.4	-8.9
40 kPa	Semi-weight bearing	-31.1	-53.0	-9.8	-43.2	-57.5	-5.4	-52.1
80 kPa	Supine	-2.1	-14.3	2.9	-17.2	-16.8	3.0	-19.8
80 kPa	Semi-weight bearing	-25.1	-99.4	-14.5	-84.9	-122.5	-12.6	-109.9
All pressures	Supine	-4.9	-13.7	2.0	-15.7	-47.3	3.0	-50.3
All pressures	Semi-weight bearing	-22.4	-37.0	-17.3	-19.7	-122.5	-5.4	-117.1

Table 5-2: The effects of applying plantar foot pressure on skin blood flow. The values represent the decrease in laser Doppler flux from the baseline values and are expressed in perfusion Arbitrary Units.

Table 5-2 illustrates the effects of applying plantar foot pressure on skin blood flow, which is shown as the reduction in laser Doppler flux from the baseline recording. All the ranges show a reduction in skin blood flow following the application of plantar foot pressure.

5.5.6 Hyperaemic response

Table 5-3 shows the time taken to reach the maximum peak hyperaemic response post release of plantar foot pressure that was applied for the duration of 3 minutes. Although the Wilcoxon paired sample test showed a statistical difference ($p<0.05$) between the two positions for all the data, closer investigation of the data reveals some large values for the interquartile and 90% ranges, with the percentiles and ranges for the application of 40 kPa in the supine position showing a similar trend to the semi-weight bearing position of higher values.

Applied Pressure	Position	Median	Percentiles		IQR	Percentiles		90% range
			25th	75th		5th	95th	
20 kPa	Supine	11.9	2.6	55.0	52.4	2.0	66.8	64.8
20 kPa	Semi-weight bearing	42.5	29.1	128.4	99.3	27.6	154.0	126.4
40 kPa	Supine	5.2	4.2	151.0	146.8	3.9	199.5	195.6
40 kPa	Semi-weight bearing	61.8	37.7	70.1	32.4	31.5	71.1	39.6
80 kPa	Supine	5.0	2.6	30.4	27.8	2.0	38.6	36.6
80 kPa	Semi-weight bearing	60.1	27.2	72.2	45	16.6	75.8	59.2
All pressures	Supine	5.2	4.0	33.8	29.8	2.0	199.5	197.5
All pressures	Semi-weight bearing	57.7	32.0	70.1	38.1	16.6	154.0	137.4

Table 5-3: The effects of the release of plantar foot pressure after 3 minutes on skin blood flow. The values show the time taken (in seconds) to reach the highest laser Doppler flux values recorded for each subject (that is, time to peak of the hyperaemic response).

Table 5-4 below shows the maximum hyperaemic response following the release of plantar foot pressure that had been applied for a period of 3 minutes. The percentiles and ranges presented indicate a consistent pattern of lower values for the supine position when compared to the semi-weight bearing position. This consistency may explain the high level of statistical significance achieve by the Wilcoxon paired sample test ($p<0.01$).

Applied Pressure	Position	Median	Percentiles		IQR	Percentiles		90% range
			25th	75th		5th	95th	
20 kPa	Supine	85.5	75.5	139.7	64.2	75.4	154.5	79.1
20 kPa	Semi-weight bearing	185.9	118.6	243.7	125.1	110.7	248.4	137.7
40 kPa	Supine	133.9	77.4	181.7	104.3	59.3	197.0	137.7
40 kPa	Semi-weight bearing	230.6	149.3	328.8	179.5	128.1	355.7	227.6
80 kPa	Supine	158.7	82.8	285.1	202.3	71.5	313.2	241.7
80 kPa	Semi-weight bearing	307.5	207.7	481.8	274.1	200.0	514.4	314.4
All pressures	Supine	124.2	75.5	186.4	110.9	59.3	313.2	253.9
All pressures	Semi-weight bearing	230.2	156.7	328.9	172.2	110.7	514.4	403.7

Table 5-4: The effects of the release of plantar foot pressure after 3 minutes on skin blood flow. The values show the maximum peak hyperaemic response (in perfusion Arbitrary Units).

Table 5-5 illustrates the peak hyperaemic response from the baseline laser Doppler flux values following the removal of the applied plantar foot

pressure that was applied for the duration of 3 minutes. The percentiles and ranges in Table 5-5 show a similar trend to the above table with higher values for the semi-weight bearing position than the supine position. In this case however the Wilcoxon pair sample test achieved a statistical difference between the two positions of $p<0.05$.

Applied Pressure	Position	Median	Percentiles		IQR	Percentiles		90% range
			25^{th}	75^{th}		5^{th}	95^{th}	
20 kPa	Supine	77.4	56.7	94.4	37.7	53.9	96.0	42.1
20 kPa	Semi-weight bearing	157.0	90.0	214.2	124.2	83.1	217.9	134.8
40 kPa	Supine	133.9	77.4	181.7	104.3	59.3	197.0	137.7
40 kPa	Semi-weight bearing	209.1	90.4	289.2	198.8	59.6	307.0	247.4
80 kPa	Supine	149.6	79.6	260.6	181	68.7	285.2	216.5
80 kPa	Semi-weight bearing	221.1	183.1	427.5	244.4	180.2	486.5	306.3
All pressures	Supine	104.2	66.1	164.7	98.6	53.9	285.2	231.3
All pressures	Semi-weight bearing	197.4	128.2	246.8	118.6	59.6	486.5	426.9

Table 5-5: The effects of the release of plantar foot pressure after 3 minutes on skin blood flow. The values show the maximum peak hyperaemic response from baseline (in perfusion Arbitrary Units).

The hyperaemic response occurred following the release of applied plantar foot pressure after a total duration of three minutes. Figure 5-1 shows a typical hyperaemic response (the fluctuating flux signal is characteristic of the laser Doppler and has been explained earlier). Note that immediately after the release of applied pressure there is a surge of blood flow resulting in the maximum peak laser Doppler fluxmeter flux. Following the maximum peak laser Doppler fluxmeter flux there is a recovery phase as the flux settles back to baseline. Overall, the maximum hyperaemic response, shown in all tables and Figure 5-7, was significantly higher in the semi-weight bearing position (median 230.2 AU) than the supine position (median 124.2 AU) ($p = 0.006$). Similarly, the peak response from baseline was elevated in the semi-weight bearing position (median 197.35 AU) compared to the supine position (median 104.2 AU) ($p = 0.028$) (see Table 5-1 and Figure 5-7). The time taken to reach the maximum peak hyperaemic response was also greater in the semi-weight bearing position (median 57.7 seconds) than the supine position (median 5.2 seconds) ($p = 0.034$) (see Table 5-1 and Figure 5-7).

5.4 Discussion

The laser Doppler fluxmeter has been shown by various authors to be a suitable instrument to measure the venoarteriolar response (Delis *et al.,* 2001; Bull *et al.,* 1995; Belcaro *et al.,* 1991; Belcaro *et al.,* 1989). Bircher *et al.* (1994) and Ingolfsson *et al.* (1994) reported on how the quality of the laser Doppler fluxmeter signal is also dependent on environmental experimental conditions and probe positioning. Engelhart and Kristensen (1983) compared the laser Doppler fluxmeter against the ^{133}Xenon technique to measure cutaneous blood flow and concluded that the laser Doppler fluxmeter not only measures capillary blood flow but also blood flow in the venoarteriolar anastomoses which is part of the temperature regulation mechanism. Thus it is vital to control the environmental temperature to reduce the thermoregulatory mechanisms affecting the results. Probe positioning is also vital for repeatability and reproducibility of results (Bircher *et al.,* 1994; Ingolfsson *et al.,* 1994). The advantage of the developed system is that the laser Doppler fluxmeter and pressure probes are held in one position within the piston mechanism. In addition, to ensure that the same area on the plantar aspect of the heel was sampled, guide lines where drawn on the shoe to allow correct alignment of the foot. Following alignment, the foot was strapped to ensure that no foot displacement occurred from the correctly aligned position.

On standing the lower limb vascular pressure increases stimulating a local sympathetic axon reflex that triggers the pre-capillaries and arterioles to vasoconstrict, referred to as the venoarteriolar response (Delis *et al.,* 2001). This hypothesis is supported by studies that have shown this vasoconstrictive response to be present after spinal sympathetic blockade but is abolished by α-adrenergic blockade or by the local injection of anaesthesia (Hassan *et al.,* 1987; Henriksen *et al.,* 1976). This compensatory mechanism exists to limit nutritional blood flow at capillary level by reducing skin blood flow with the purpose of reducing the formation of oedema in the dependant limb (Delis *et al.,* 2001; Flynn *et al.,* 1989; Gaswell *et al.,* 1971). This mechanism is independent of the neurogenic mechanism that controls blood flow through the venoarteriolar shunts (Flynn

et al., 1989). A study was carried out to investigate the effect of the veno-arteriolar response on the foot and sympathetic release purely on the capillary component of peripheral cutaneous blood flow. Cutaneous microcirculation was studied in the supine and near standing position before and after indirect heating, which was carried out by warming the body trunk to 44°C and measuring skin blood flow in the nailfold. The study showed that during indirect heating the percentage fall in capillary blood flow associated with dependency remained unchanged suggesting that a compensatory mechanism exists to limit nutritional skin blood flow by controlling both red blood cell velocity and duration of flow. That is, in addition to the sympathetic tone there is a local vasoconstrictor mechanism that operates in the vascular resistance elements in closest proximity to the capillary bed and distal to the resisting elements controlling the venoarteriolar shunts (Flynn *et al.,* 1989). However, our study into the effects of plantar foot pressure on skin blood flow suggests that following a period of applied pressure and local starvation, the local vasoconstrictor mechanism is overridden resulting in vasodilatation and a greater hyperaemic response with the leg in dependency than in supine. These were observed during the application of 20, 40 and 80 kPa of plantar foot pressures to the center of the heel. However, due to the low number participating in the study more research is needed to confirm this finding.

The venoarteriolar response physiological mechanism causes a rise in pulse rate, reduces cardiac output and the velocity of venous blood flow in the lower limbs, limits the increment in capillary pressure, reduces blood flow and allows intracapillary osmotic pressure to rise and oppose the continued transcapillary filtration of fluid in the dependant leg (Delis *et al.,* 2001; Flynn *et al.,* 1989; Beaconsfield *et al.,* 1955). In patients with diabetes mellitus and peripheral vascular disease failure of the venoarteriolar response has been found. In patients with diabetes mellitus and patients with intermittent claudication the venoarteriolar response was diminished, however in patients with resting pain and impending gangrene the venoarteriolar response was completely abolished (Delis *et al.,* 2001; Belcaro *et al.,* 1991; Belcaro *et al.,* 1989). This may explain why patients

with intermittent claudication find that by assuming a sitting posture with the legs dangling or walking the ischaemic pain is relieved. This may be due to the fact that failure of the venoarteriolar means that when in an upright posture failure of the reflex vasoconstriction causes an increase in arterial blood pressure and an increase in blood flow to the legs. This increase in blood flow is sufficient to replenish the deficit in tissue oxygen and the relief of the ischaemic pain. However such hypothesis is beyond this thesis, future studies should be conducted.

Significant statistical differences were found between baseline blood flow with the subject in a supine and semi-weight bearing positions. Following the application of pressure the decrease in flux values from baseline was greater in the semi-weight bearing position when compared to the supine position and were statistically significant. It appears that the greater decrease in total flux for semi-weight bearing values suggests that applying plantar foot pressure to a vascular bed, in the plantar aspect of the heel, which is already subject to postural vasoconstriction seems to cause a greater reduction in cutaneous blood flow. A future study should investigate this in more depth since this is beyond the aim of this study and thesis.

Previous studies have shown a difference between flux values in the supine position and in dependency in areas with and without venoarteriolar shunting (Flynn *et al.,* 1989; Hassan *et al.,* 1986). In addition, in areas of skin where venoarteriolar shunts are numerous, the reduction in cutaneous blood flow on dependency was abolished by the release of sympathetic tone (Flynn *et al.,* 1989). Since the laser Doppler fluxmeter partly measures venoarteriolar shunts and the soles of the feet are rich in venoarteriolar shunts, our results are partly affected by this and do not solely record nutritional blood flow. This study's reactive hyperaemic responses reflect both, the nutritive blood flow and effects on arteriovenous shunts following pressure ischaemia.

Reactive hyperaemia is a manifestation of the repayment local blood flow regulation mechanism that is set into motion after a period of vascular occlusion and lasts long enough to repay the tissue oxygen and nutrient

deficit that accrued during the vascular occlusion period. The mechanism emphasizes the close relationship between local blood flow regulation and supply of nutrients to the tissues (Engelhart *et al.*, 1983; Guyton, 1981). Thus it may reflect the level of insult to the soft tissues with the greater the insult the greater and longer the hyperaemic phase and vice versa. The study suggests that differences exist between measuring the hyperaemic response following pressure ischaemia in a supine and semi-weight bearing position with the semi-weight bearing position reaching high hyperaemic response values. It may be that gravity may be related in producing a quicker and larger hyperaemic response in the foot when the subject is upright as compared to the subject in a supine position where the effects of gravity on blood flow to the foot is minimised. In addition, the higher the pressure applied in both positions the greater the hyperaemic response found (see Table 5-1). Meinders *et al.* (1996) found similar findings with a greater hyperaemic response with increasing pressures of 20, 40 and 80 kPa applied to the center of the heel in the supine position only.

5.5 Conclusion

The aim of this thesis is not to provide an in-depth understanding of the effects of plantar foot pressure on skin blood flow in relation to the postural control but to investigate whether a difference exists between laser Doppler fluxmeter measurements taken in the supine or semi-weight bearing positions and this was achieved. Thus the study investigating the effects of plantar foot pressure on skin blood flow in patients with rheumatoid arthritis will be carried out with subjects in a semi-weight bearing position where the venoarteriolar response is active and the physiological responses are more representative of human walking stance (that is, an upright posture).

Future studies investigating the effects of plantar foot pressure on skin blood flow should carry out their investigating with the subjects in an upright position mimicking the vertical posture taken during human walking. Similarly, tissue viability studies investigating the effects of external pressure on skin blood flow for bed-ridden patients should carry out their investigations with the subjects in the supine position. In addition, since the

responses between the positions described for the effects of external pressure on skin blood flow vary, only the results of published articles with the subjects in the same position may be comparable. Finally, in the light of differences between physiological vascular responses to the application of plantar foot pressure between both measuring positions, more research should be carried out in future.

5.6 Limitations and recommendations

This study raises a number of important issues in the field of tissue viability. If differences exist between vascular responses to applied pressure in the two positions investigated then the development of soft tissue breakdown (that is, ulceration) is not only dependant on the amount of pressure applied, anatomical location, etc., but also in the position of the subject. For example, for studies investigating the development of soft tissue breakdown in the sacral area, the studies should be conducted with the subject in a supine position; for studies investigating soft tissue breakdown over the Gluteus Maximus muscle, the studies should be carried out with the subject supine if investigating bed ridden patients or in the sitting position if investigating wheel chair bound patients; finally, for the investigation of walking related soft breakdown in the plantar aspect of the foot subjects should be in an upright position. Thus, future published articles should state clearly the measuring position and making general assumptions of soft tissue breakdown between tissue viability articles conducted in various positions may not be possible due to different vascular physiological responses to applied pressure that is related to subject position. This thesis recommends that future research should be carried out regarding the vascular response of soft tissue to applied pressure in various positions.

It also recommends that more *in vivo* validation of the laser Doppler fluxmeter should be carried out.

CHAPTER 6

MODIFICATIONS AND FURTHER VALIDATION OF DEVICE

6. CHAPTER 6: Modifications and further validation of device

6.1 Introduction

In Chapter 4 a system to measure the effects of plantar foot pressure on skin blood flow was developed and validated. However, recommendations where made regarding improving the device, since the previous system was visually/sound synchronised and this introduced a time delay error into the system. Other recommendations were also made (see section 4.7).

In this chapter a synchronisation box was developed which allowed the laser Doppler fluxmeter (DRT4) to be electronically synchronised in real time with the Novel standard system using a Novel Pliance-M Expert capacitative pressure transducer. Synchronising these two widely used pieces of equipment allows access to research into this recent area of study investigating the effects of externally applied pressure on skin blood flow with minimal bioengineering equipment development and promoting new research.

The shoe device was also modified to include a "control" transducer for the laser Doppler fluxmeter in the forefoot and the diameter of the three-tier piston was reduced. A hand-held electronic analogue marker was also developed to mark events other than the study protocol periods. The aim of this chapter was to improve the developed system to measure the effects of plantar foot pressure on skin blood flow and validate the device following the modifications.

6.2 System modifications and additions

6.2.1 Modifications to measurement shoe and pressure system

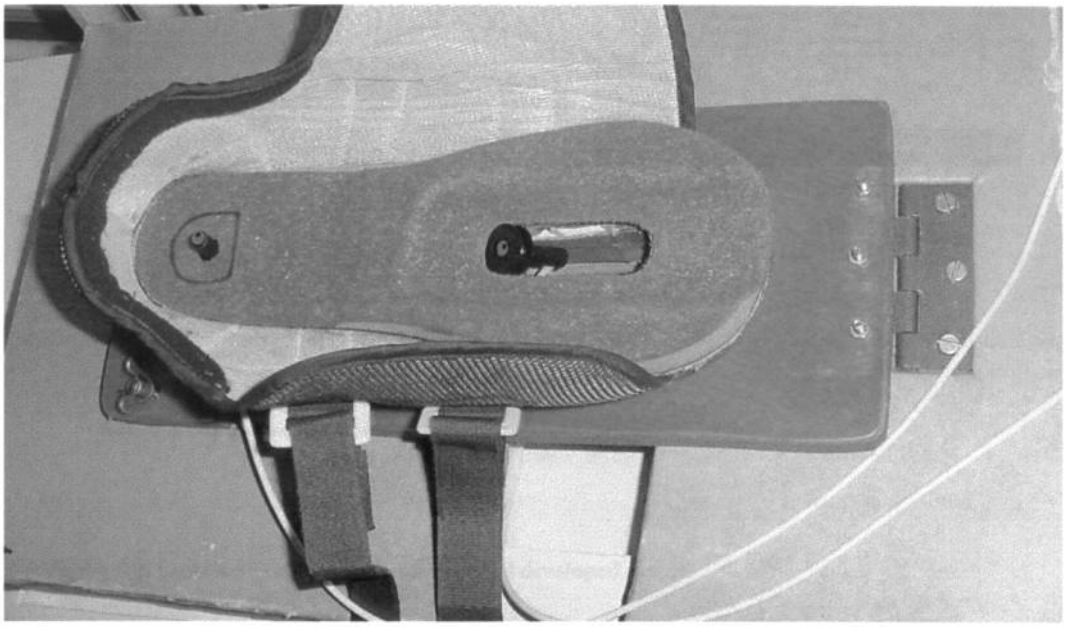


Figure 6-1: The modified measurement shoe. Note the reduced diameter piston at the centre of the heel and the groove to accommodate the control probe that was stuck to the skin with double-sided adhesive tape.

The measurement shoe was modified to fit the reduced diameter three-tier piston and the control probe at the forefoot (see Figure 6-1 and Figure 6-2). The piston diameter was reduced by removing the laser Doppler fluxmeter transducer housing and making the top tier of the three-tier piston the exact size of the transducer (type DP1T/7-14). Thus, the area where pressure is applied on the skin is the area sampled by the skin blood flow-measuring instrument.

The strain gauge was replaced with a custom made Pliance-M Expert capacitive transducer (Novel, Germany)(0.5 mm in thickness) to make the middle tier of the piston. The new piston system consisted of the pressure transducer connected to the Pedar Standard Pliance box (see Figure 6-2, Figure 6-9 and Figure 6-7). The Pedar Standard Pliance box is connected to the Multi IO interface box where the unit is powered by a transformer. The Multi IO interface box connects to a computer for data capture.

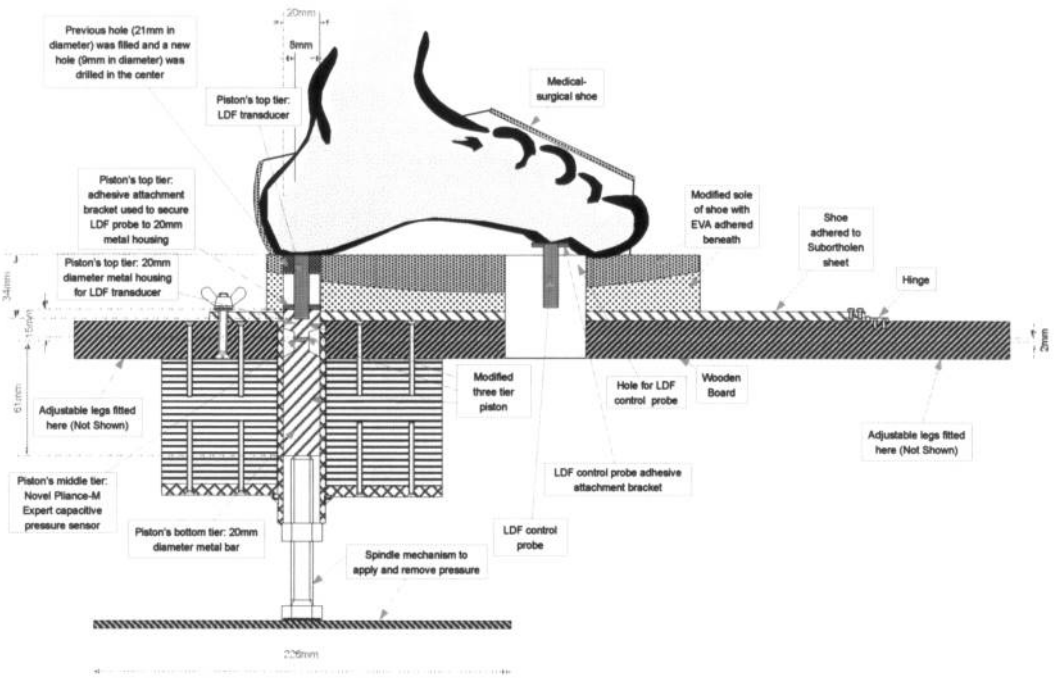


Figure 6-2: Cross-sectional drawing of modified developed device.

The hole 2.1 centimetres in diameter was filled in with resin glue and an ethyl vinyl acetate plug[5] and a new hole 9 millimetres in diameter was drilled in the centre of the heel. This accommodated the top section of the three-tier piston (that is, the laser Doppler fluxmeter transducer 8 millimetres diameter by 34 millimetres high). Turning the spindle mechanism (described in section 4.2.3.1) allowed the reduced diameter piston to move up and apply plantar foot pressure or down and remove the applied pressure (see Figure 6-2).

A groove 2.2 centimetres by 7 centimetres was cut in the forefoot to accommodate the laser Doppler fluxmeter transducer "control" probe (that is, a site on the forefoot where no plantar foot pressure was applied throughout the measurement protocol). The groove allowed for the "control" skin blood flow probe to be placed on the centre of the 3rd metatarsal head for any foot size. This allowed for speed, concentration and flux of moving red blood cells to be collected at the "control" site (see Figure 6-2).

[5] Filling the hole in the centre of the heel and drilling a new smaller hole did not affect the structure of the shoe and facilitated operation of the modified piston.

6.2.2 Development of an electronic marker device

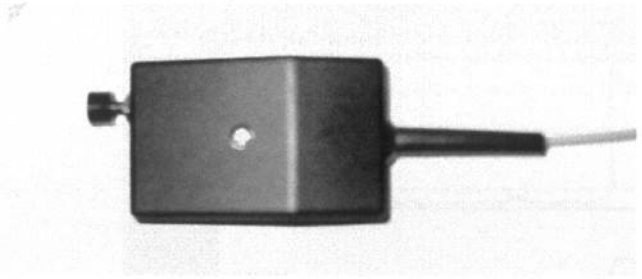


Figure 6-3: The electronic marker with the push button (left of device) and cable exiting (right of device)

An electronic marker was constructed to record any events (other than the protocol markers) on the laser Doppler fluxmeter trace. Events that were recorded were the pressure application and removal periods and any other artefact events that could affect a part of the trace (for example, a brief cough). These small events could then be excluded from the analysis.

The marker was encased in a small plastic box that could fit in the palm of the hand and powered by a 1.5-volts battery with an on/off switch that was fitted to the box (see Figure 6-3). The cable, using a Bayonet Nut Connector (BNC), was connected to the analogue input connector of the laser Doppler fluxmeter AN02 box that in turn was connected to the DRT4 unit. During data collection a 1.5-volt impulse was used to mark events on the skin blood flow traces (see Figure 6-4). The protocol events marker set-up using the iontophoresis software (explained in section 4.2.5) can also be seen in Figure 6-4).

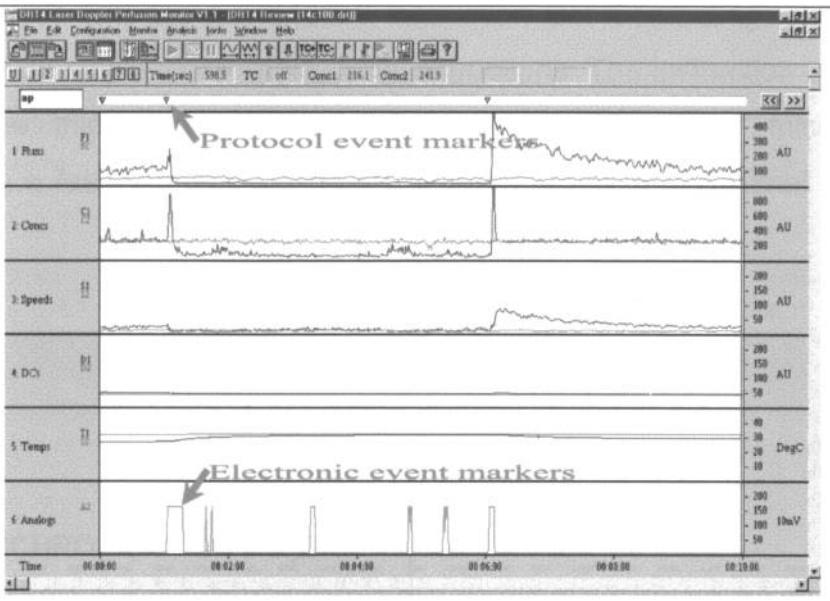


Figure 6-4: The laser Doppler fluxmeter trace. Trace 1 to 3 shows the blood cell flux, concentration and speed in perfusion units. Trace 4 shows the background light (DC) and trace 5 the skin temperature. Finally, trace 6 shows the analogue 1.5 volts electronic markers indicating when the event happened and the duration. The protocol electronic markers are indicated on the top white line as gray triangles.

6.2.3 Development of an electronic synchronisation method

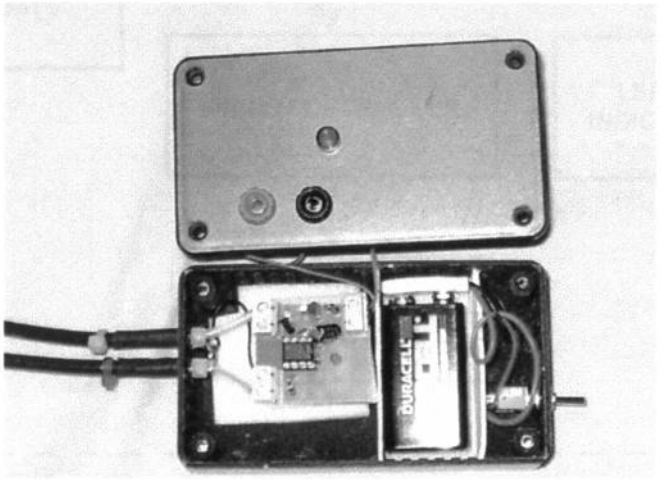


Figure 6-5: The Custom-made synchronisation box for the Novel Pliance-M Expert pressure system and the laser Doppler fluxmeter system. Note the printed circuit board with the micro-controller and LED on the left side of the box. The right side contains the 9-volt battery and on/off switch.

The custom-made synchronization box was designed and developed in the Foot Pressure Analysis Laboratory (Institute of Motion Analysis and Research, Department of Orthopaedic and Trauma Surgery, University of Dundee, Dundee, Scotland) and is based around a small micro-controller using Microchip Technology. The device is a low-cost high performance 8-

bit controller employing 'Reduced Instruction Set Computing' architecture with only 33 single word/single cycle instructions. The instruction cycle time using the micro-controllers own onboard oscillator is 1 microsecond providing a fast real time synchronization capability with minimal sampling latency.

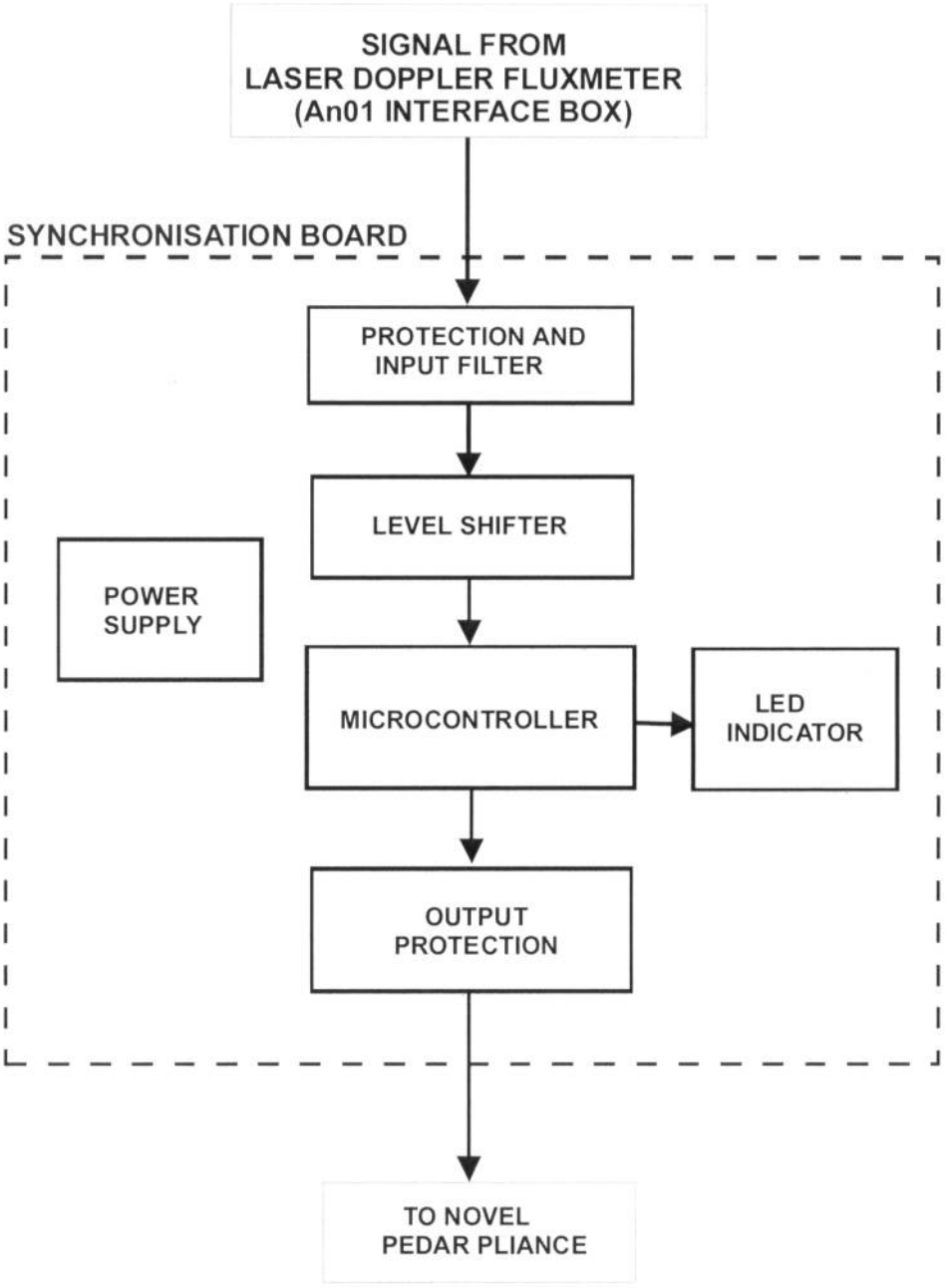


Figure 6-6: Schematic of the synchronisation box circuit.

The synchronization box circuit was constructed on to a custom-made printed circuit board, mounted in a small plastic enclosure. The enclosure also contains 9-volts battery used to power the synchronization box, an

on/off switch, and two connections; one to the laser Doppler fluxmeter AN02 interface box and the other to the Novel Pliance-M Expert pressure system. A battery was used in the design to enable portability of the synchronization box and isolate participants from mains power (see Figure 6-5).

The printed circuit board contains the micro-controller, power supply section, signal conditioning and protection circuitry to provide fault tolerant interfaces to the laser Doppler AN02 box and the Novel Pliance-M Expert systems (see Figure 6-6). An LED was fitted to provide a visual feedback of the synchronisation operation.

5.5.7 Signal processing by synchronisation box

Upon starting the laser Doppler fluxmeter iontophoresis software (see section 4.2.5) a 2.49-Volts output signal is generated via the AN02 box (see Figure 4-9). This signal is received and used by the synchronisation box to trigger the Novel pressure system to commence recording. This is achieved by the synchronisation box converting the 2.49-Volts single input signal received from the laser Doppler fluxmeter AN02 box into a pulsed 5-Volts output signal that is sent to the Novel multi IO interface.

The circuit in the synchronisation box consists of an input filter to reduce high frequency signal to noise and provide protection. A level shifter is used to convert the typical single 2.49-volt pulse from the laser Doppler fluxmeter into a 5-volt pulse. The output circuits provide buffering and current limited outputs between the micro controller and the external connection to the Novel Pedar Standard system. This provides a fault tolerant circuit protecting both the synchronisation box micro controller and the Novel Pedar Standard system interface.

When the synchronisation box is switched on, the micro controller executes its initialisation program module. This loads the onboard oscillator calibration data, initialises the internal memory and sets up the input/output port. It then executes the start loop program module that constantly monitors the input signal from the laser Doppler AN02 box waiting for the rising edge of the first pulse. This pulse starts the data acquisition program

module code, which then outputs regular pulses to the Novel Pedar Standard system. Each output pulse instructs the Novel Pedar Standard system to take a single pressure sample. These pulses provide a maximum foot pressure-sampling rate of 40 readings per second. A second 2.49-Volts pulse from the laser Doppler AN02 box terminates the data acquisition program module.

The start loop program module has a program loop time of 6 microseconds with the start pulse rising edge test taking 2 microseconds. The data recording start latency is therefore between 2 and 6 microseconds depending where in the start loop the pulse arrives.

5.5.8 Synchronised data collection method

The experiment protocol (refer to section 4.2.5) was set-up using the DRT4's iontophoresis software so that a start signal of 2.49 volts is transmitted at the start; with several automatic markers of 0 volts and a final signal of 2.49 volts to stop the Novel pressure system. The DRT4 iontophoresis program was then transmitted to the DRT4 from the computer before the start of data recording.

Using the Novel Pliance-M Expert standard online software, the system is set to slave and the frames per second are entered. The record button is then pressed with the pressure sensor unloaded followed by the play button. The system is now ready to receive the synchronization signal to start.

With the foot in the specially constructed shoe, skin contact is made by the laser Doppler fluxmeter probe. Skin blood flow recording is started using the DRT4 software; upon pressing the iontophoresis start button the study data collection protocol is started, with the DRT4 via the AN02 box triggering the synchronization box, which in turn starts the Novel pressure system in real time.

6.3 Overview of modified system

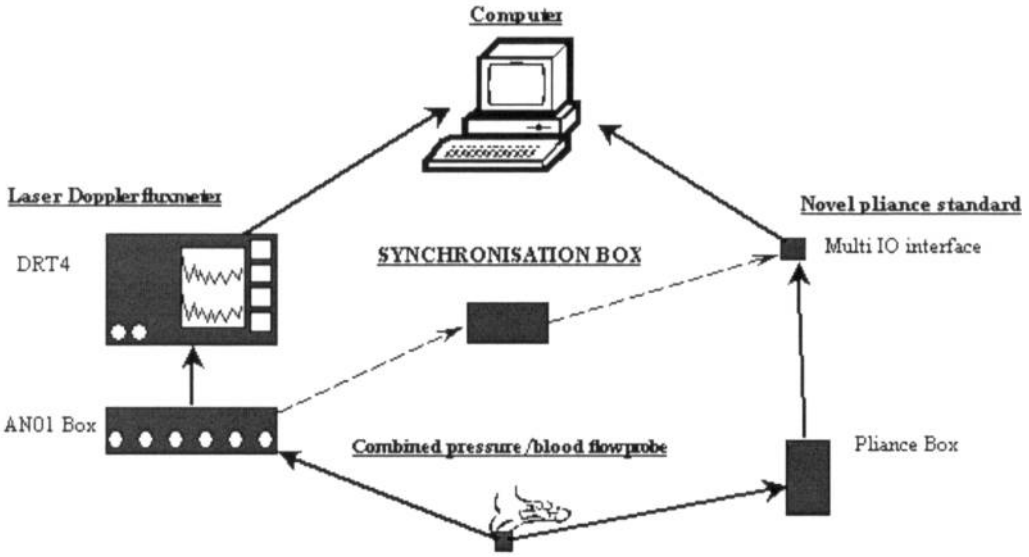


Figure 6-7: Communication between the various devices. On starting the iontophoresis program within the DRT4 the out signal from the AN01 box triggers the synchronisation box to start recording plantar foot pressure. At the end of the iontophoresis program a second signal stops the pressure recording.

The Novel Pedar Standard system allows for the measurement of plantar foot pressure using a custom-made capacitive sensor. The system consists of a standard box, which electronically receives the sensor's load signal and transmits it to the multi IO interface box that in turn sends the signal to the computer. The system allowed for the synchronised measurement of skin blood flow and plantar foot pressure.

6.4 Calibration

6.4.1 Novel pressure system calibration

6.4.1.1 Novel capacitive sensor calibration

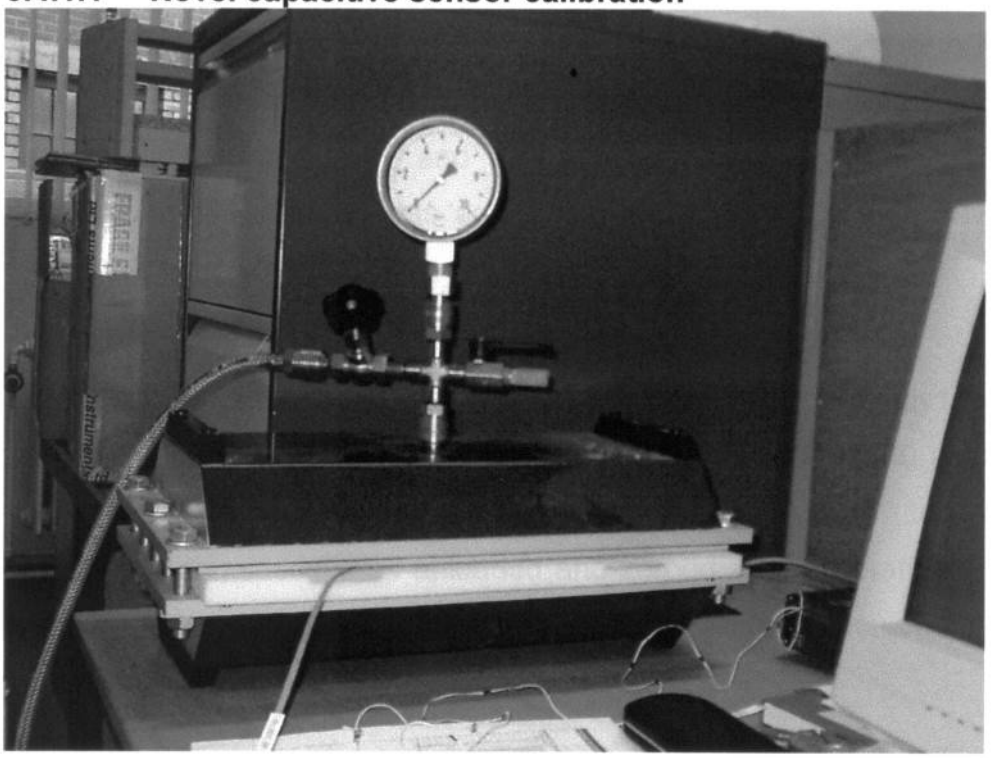


Figure 6-8: The Novel calibration system where pressure is applied to the capacitive sensor using a thin rubber balloon bladder.

The first stage of the calibration involved producing a calibration file. The final stage involved producing a routine calibration curve at known pressures. The transducers should be routinely calibrated using the users hardware as the manufacturers have found variations using the same transducer on different hardware set-ups.

6.4.1.2 Novel calibration vice

This consists of a calibration vice (see Figure 6-8) with a white calibration board covered in leather. The transducer is place in the centre of the board with the serial number facing up. The white board was slid into the metal chamber. During the calibration program air is pumped, from a compressor, to the metal chamber where a rubber bladder is inflated which produces a uniform pressure over the transducers (8mm diameter) resting on the white board. The pressure is quantified using a pressure gauge.

6.4.1.3 STAGE 1: Setting calibration files using the Iterativ method

Using the "Pliance settings" software a range of 0 to 200 KPa was recorded[6]. A pressure was applied to the transducer and amplification set. The pressure was released and after a time lapse of 20 seconds (to allow the transducer to relax) the offset values were set and the system tested. The process was repeated 5 times. Amplification A/D value of 239 (240 ±5 recommended by Novel) and an offset A/D value of 30 (30 ±5 recommended by Novel) was achieved. The tables were then saved as filename "ra_study1.tbl".

6.4.1.4 STAGE 2: Calibration procedure

Using the "calibration" software and the table file "ra_study1.tbl" the calibration steps were defined and saved (that is, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200 kPa). Using the Novel calibration vice (see section 6.4.1.2) the transducer was unloaded and zeroed. The calibration process was then started and consisted of loading the pressure to each of the above values and saving the data.

The calibration lines window was then used with the saved data to produce a calibration line. The manufacture recommend that the extrapolated line should start at zero, end at the highest set range and have a slight bend. Failure to achieve that curve means that the calibration process is not valid and has to be repeated. The study's calibration line achieved the manufacture's recommendations and the calibration files were saved.

Novell recommends a calibration check of the transducer every three months by applying known pressures to the transducer and comparing with the pressure obtained from the online measurement. This is advised for frequent use of transducers as the cells may take permanent deformation over time. In the current study the transducers were used only for the purpose of the study and hence for light use. Thus, a three months check is more than ample. Following the calibration of the transducer it was

[6] A range of 0 to 200 kPa was selected to allow the researcher some flexibility for determining in the later study suitable pressures to be applied to the rheumatoid arthritis subjects that would not cause pain or place subjects at risk of soft tissue breakdown.

immediately tested and retested every three months until the end of all the data collection.

6.4.2 Laser Doppler fluxmeter calibration

The calibration of the laser Doppler fluxmeter DRT4 was checked as described in section 4.3.1 using Brownian motion of latex particles in suspension. No re-calibration of the equipment was necessary since the flux and concentration of measurements were within the recommended limits.

6.5 Construction of a device to validate the pressure sensor in-shoe

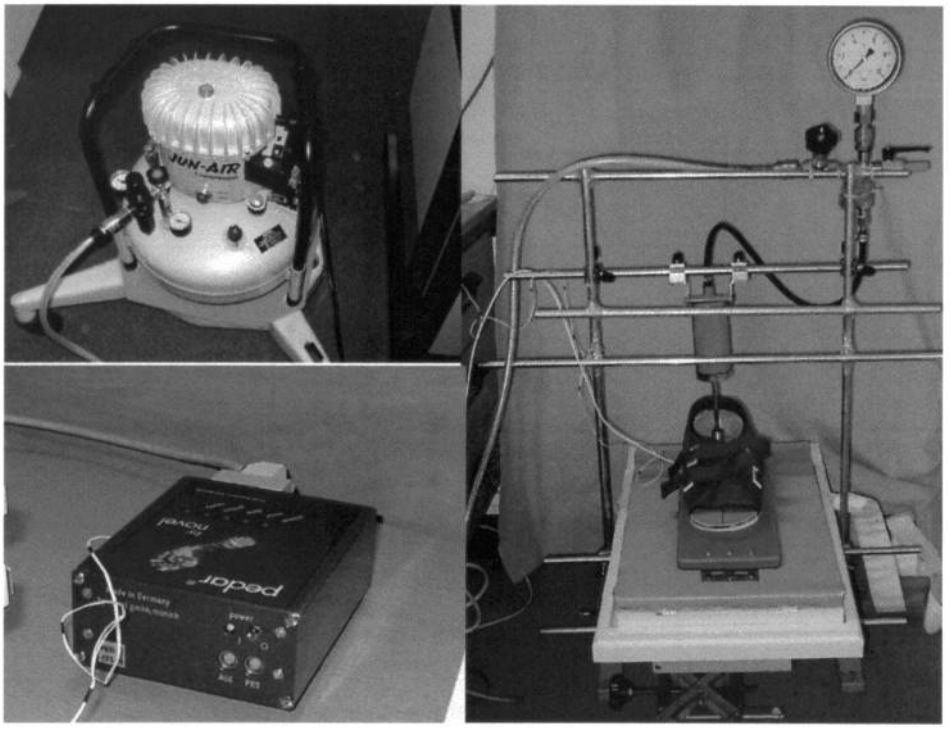


Figure 6-9: The constructed validation device to test the Novel Pliance-M Expert pressure transducer (right picture). The compressor used to pump air into the pneumatic piston (top left picture) and the Novel Pedar Pliance box where the capacitive pressure transducer was connected (bottom left picture).

To validate the Novel Pedar Standard plantar foot pressure system in the measurement shoe device, within the three-tier piston, a validation device was constructed. The validation device consisted of a frame where a pneumatic piston was attached (see Figure 6-9). The tip of the piston was constructed of medium density ethyl vinyl acetate to provide a level interface with the measurement shoe's three-tier piston so that uniform pressure could be applied following positioning and alignment of the

validation device. The frame was attached to the measurement shoe in the semi-weight bearing frame. Opening a ball valve and allowing compressed air to enter the pneumatic piston applied pressure to the capacitive transducer. The exact level of pressure was read using a calibrated gauge and the air intake valve closed. Opening a second ball valve and releasing the compressed air from the pneumatic piston removed the pressure.

6.6 System validation studies

Both systems were validated within the measurement shoe device. A quick reference guide for identifying the validation experiments is provided in Table 6-1.

Experiments	*Novel pressure system*	*Laser Doppler fluxmeter*
Linearity	6.6.1.1 Study 14: Linearity of pressure measurements	Not applicable
Accuracy	6.6.1.2 Study 15: Accuracy of pressure measurements	Not applicable
Repeatability	6.6.1.3 Study 16: Repeatability of pressure measurements	6.6.2.1 Study 21: *In vivo* repeatability of laser Doppler fluxmeter measurements
Equipment warm-up	6.6.1.4 Study 17: Reproducibility of pressure measurements during equipment warm-up	Not applicable
Day to day variation	6.6.1.5 Study 18: Reproducibility of daily pressure measurements	6.6.2.2 Study 22: *In vivo* reproducibility of daily laser Doppler fluxmeter measurements
Hysteresis	6.6.1.6 Study 19: Hysteresis effects on pressure measurements	Not applicable
Range, limit of detection and quantification	6.6.1.7 Study 20: Range, limit of detection and limit of quantification of pressure measurements	Not applicable

Table 6-1: Quick reference guide to locate the validation experiments for the Novel pressure system and the laser Doppler fluxmeter.

6.6.1 Validation studies of the Novel pressure measurement system

The aim of the Novel plantar foot pressure studies was to evaluate the effectiveness of the pressure system to measure plantar foot pressure within the measurement shoe device. All measurements were taken with the Novel transducer attached to the three-tier piston within the shoe device. Quantifiable pressure was applied using the validation device explained in detail in section 6.5 in this chapter. Pressure was applied to the transducer using compressed air and a pneumatic piston. The equipment

was tested for loading and unloading, before and after dismantling, transportation and reassembly. The system was investigated for linearity, repeatability, hysteresis and reproducibility during equipment warm-up and over a five-day period. The unit of measure used for all the pressure validation experiments was bar since the calibrated manometer gauge used for the application of pressure was in bars. Where possible the values have been converted and also expressed as kilo-Pascals.

6.6.1.1 Study 14: Linearity of pressure measurements

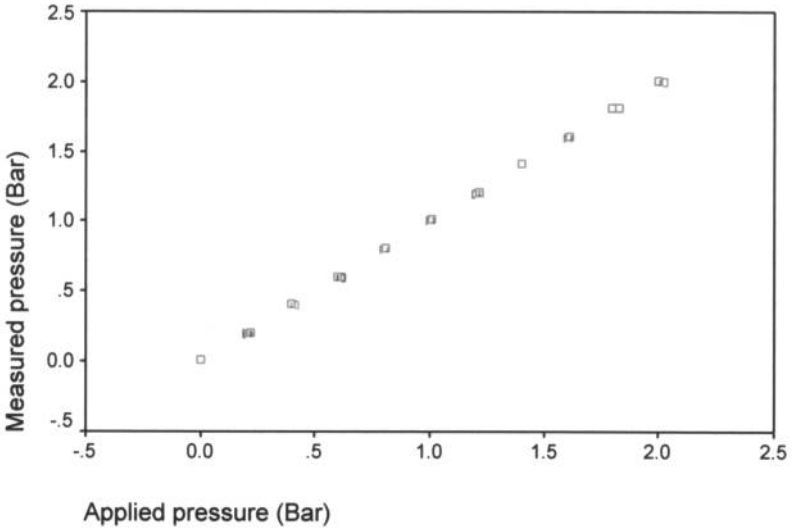


Figure 6-10: Scattergraph of 5 test re-test results for applied pressure against measured pressure for loading of the Novel pressure system for before dismantling, transportation and reassembly of the equipment. All pressures are in Bar (1 bar ~ 100 kPa).

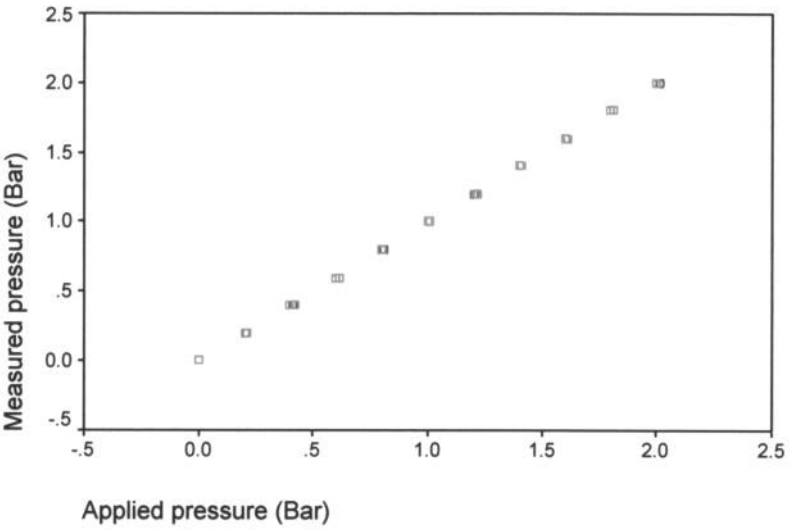


Figure 6-11: Scattergraph of 5 test re-test results for applied pressure against measured pressure for unloading of the Novel pressure system for before dismantling, transportation and reassembly of the equipment. All pressures are in Bar (1 bar ~ 100 kPa).

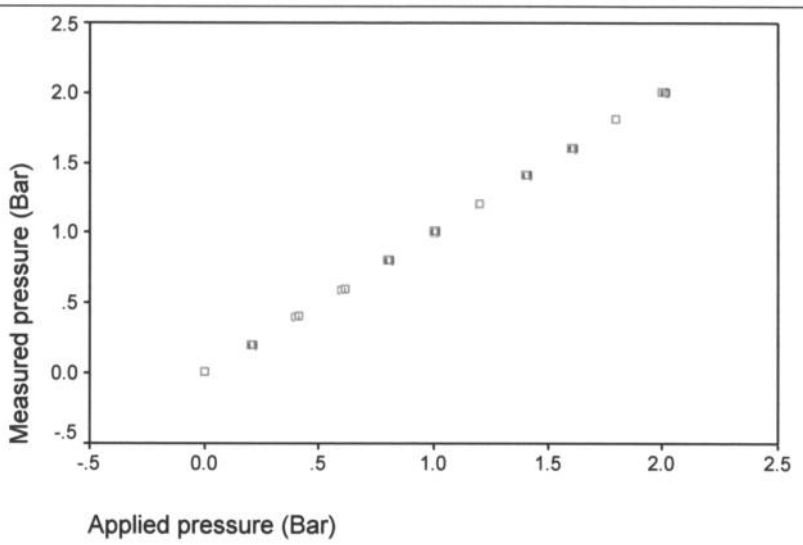


Figure 6-12: Scattergraph of 5 test re-test results for applied pressure against measured pressure for loading of the Novel pressure system for after dismantling, transportation and reassembly of the equipment. All pressures are in Bar (1 bar ~ 100 kPa).

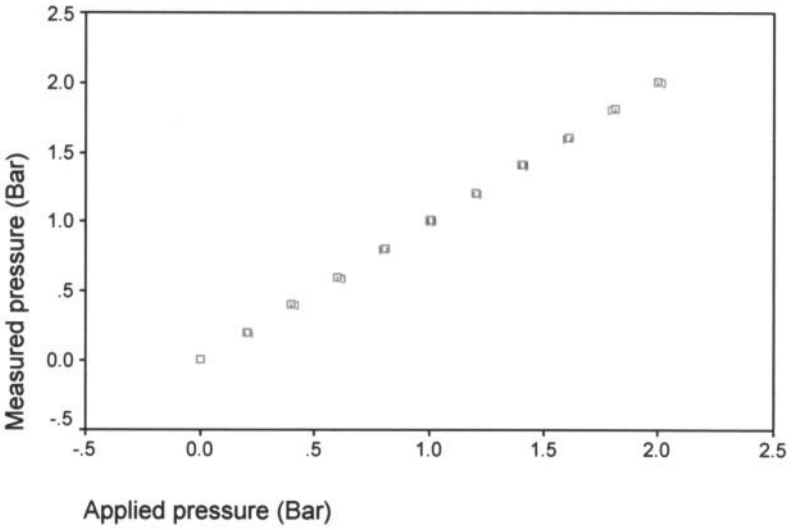


Figure 6-13: Scattergraph of 5 test re-test results for applied pressure against measured pressure for unloading of the Novel pressure system for after dismantling, transportation and reassembly of the equipment. All pressures are in Bar (1 bar ~ 100 kPa).

The importance of investigating linearity has been described in section 4.3.4. The Novel pressure system was validated for both loading and unloading. Figure 6-10 to Figure 6-13 show the linearity of the pressure system for loading and unloading over a range of 0 to 2 bar (200 kPa) of applied pressure for 5 repeated measurements both before and after moving the equipment. In all graphs the x-axis represents the applied loads and the y-axis the measured loads. The scatterplots above show a high level of linearity between the Novel pressure measurements and the known applied pressure. Loading and unloading and dismantling, transportation

and reassembly of the equipment did not affect the linearity of the pressure measurements.

	Loading		Unloading	
	R^2	Significance	R^2	Significance
Before transportation	0.99	P<0.01	0.99	P<0.01
After transportation	0.99	P<0.01	0.99	P<0.01

Table 6-2: Regression analysis for the proportion of variance accounted for by the regression and the significance levels for a regression ANOVA.

For loading and unloading, for before and after, the equipment did not affect the linearity of the system since the R^2 values are high and equal, with 99% of all the applied pressure accounted by the pressure system (see Table 6-2). In addition, the results presented are highly significant with p values for a regression ANOVA below 0.01.

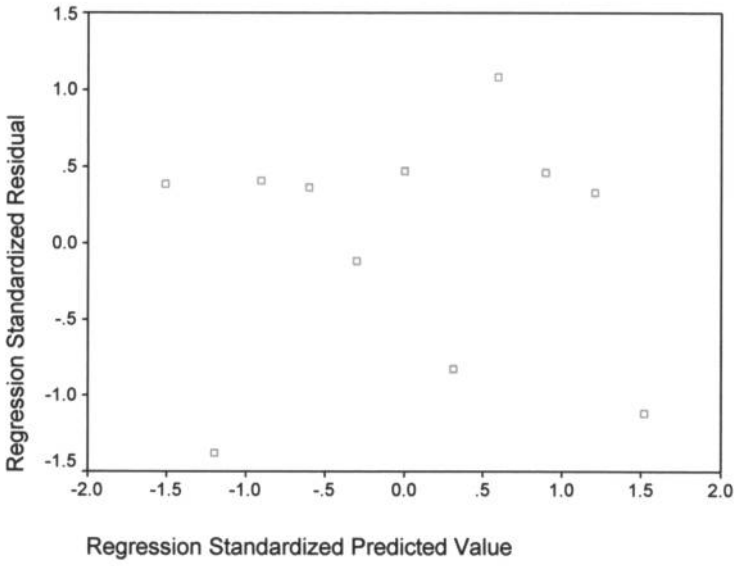


Figure 6-14: Scatterplot of the standardised residuals against standardised predicted scores for applied pressure during loading before dismantling, transportation and reassembly of the equipment.

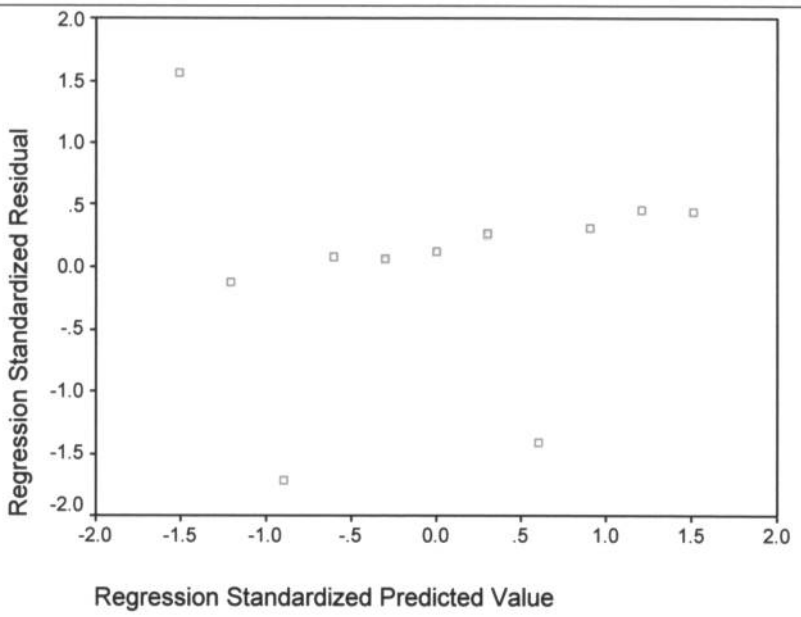


Figure 6-15: Scatterplot of the standardised residuals against standardised predicted scores for applied pressure during loading after dismantling, transportation and reassembly of the equipment.

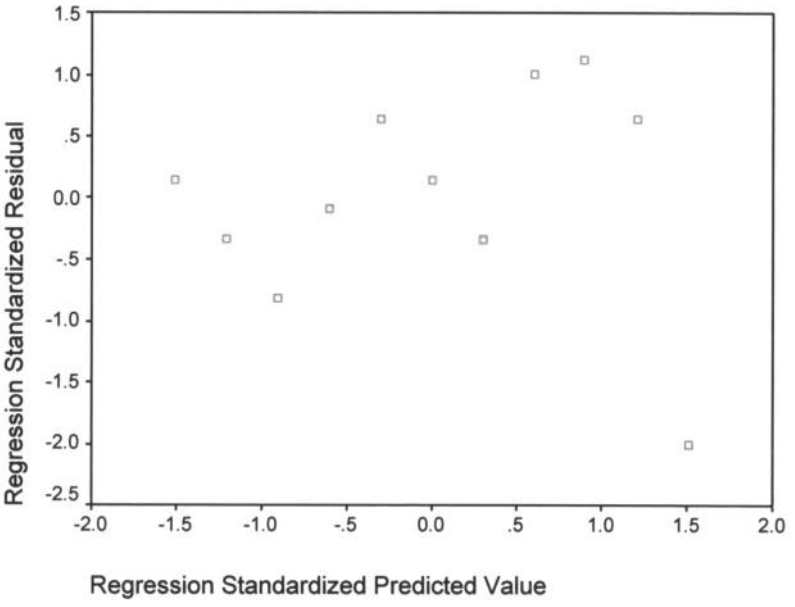


Figure 6-16: Scatterplot of the standardised residuals against standardised predicted scores for applied pressure during unloading before dismantling, transportation and reassembly of the equipment.

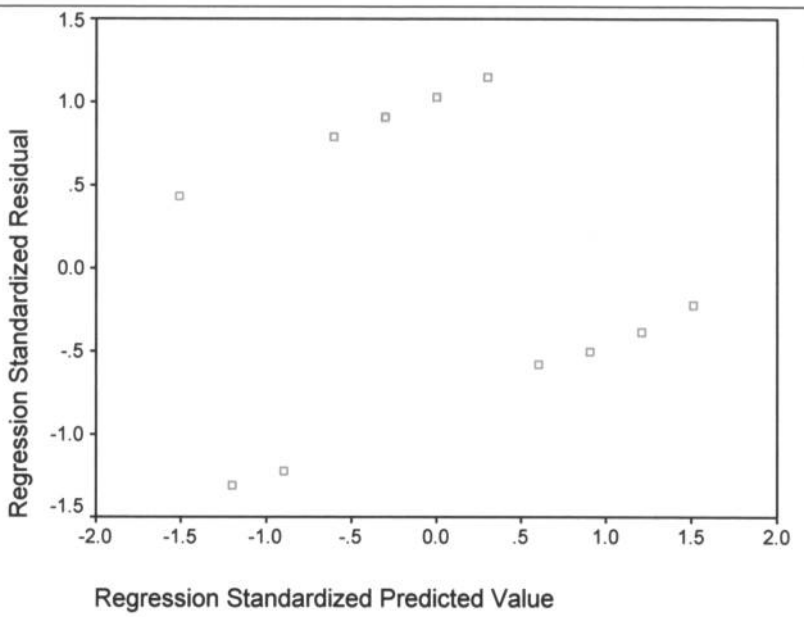


Figure 6-17: Scatterplot of the standardised residuals against standardised predicted scores for applied pressure during unloading after dismantling, transportation and reassembly of the equipment.

The x-axis represents the regression standardised predicted value and the y-axis the regression standardised residual for loading and unloading of the transducer for both before and after transportation of the equipment (see Figure 6-14 to Figure 6-17). Since none of the scatterplots show any obvious patterns the assumption of linearity and homogeneity of variance is confirmed for all factors investigated. In addition, to investigate the accuracy of the regression equation the standard error and maximal residual or non-linearity error was investigated (see Table 6-3). For loading and unloading for both before and after moving the equipment, the standard errors of the estimate and the maximal residual were found to be low indicating a high degree of accuracy for the regression line with the predicted values showing a similar trend to the regression line.

	Before transportation		***After transportation***		***Mean***
	Loading	***Unloading***	***Loading***	***Unloading***	
Standard error	0.006 (0.6)	0.003 (0.3)	0.003 0.3)	0.003 (0.3)	0.004 (0.4)
Maximal residual	0.006 (0.6)	0.003 (0.3)	0.005 (0.5)	0.003 (0.3)	0.004 (0.4)
Non-linearity error	0.30%	0.15%	0.25%	0.15%	0.20%

Table 6-3: Accuracy of the regression equation for linearity. The standard error of the estimate, maximal residual and non-linearity error for the regression equation are shown. All values are in Bar (kilo-Pascals).

6.6.1.2 Study 15: Accuracy of pressure measurements

A Bland/Altman plot was constructed to investigate the overall variability of the measured pressure values against the known applied pressure values. For the validation experiments the limit of agreement was 0.008 bar (0.8 kPa) above and -0.014 bar (1.4 kPa) below the mean difference of -0.003 bar (0.3 kPa). At a 95% confidence interval the repeatbility of the system (indicated by the coefficient of repeatability) is ±0.011 bar (1.1 kPa) or ±0.55% of full-scale deflection for the range of 0 to 2 bar (200 kPa). For the Bland/Altman plot below a correlation coefficient was carried out to determine any associations between the difference and average of applied and measured pressure. A poor correlation was found between the difference of the applied and measured pressure and average of the two variables mentioned ($r = 0.1$; $p<0.05$). This poor correlation may be related to the sequence of increasing pressure that was applied to the Pedar sensor.

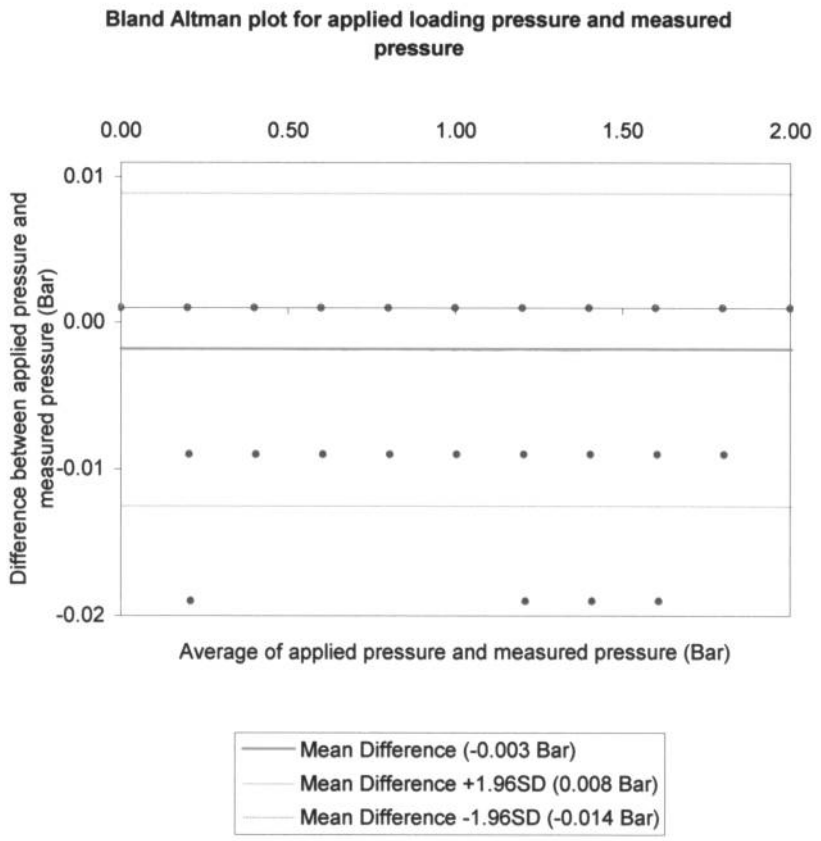


Figure 6-18: The Bland/Altman plot shows the mean and 95% limits of agreement for all the data collected during the validation of the equipment (n=616).

6.6.1.3 Study 16: Repeatability of pressure measurements

	Coefficient of Variation
BEFORE TRANSPORTATION	
Loading	0.67%
Unloading	0.55%
AFTER TRANSPORTATION	
Loading	0.41%
Unloading	0.50%

Table 6-4: The effects of test re-test on typical errors produced by the pressure system for loading and unloading, before and after dismantling, transportation and reassembly of the equipment. All values are in Bar (kilo-Pascals).

Repeated measurements taken before and after relocation and reassembly of the pressure system did not have a significant effect on the typical errors that arise when test re-test measurements are taken (see Table 6-4). This suggests that pressure measurements taken with the Novel Pliance-M Expert system are highly repeatable since the values for the coefficient of variation are low.

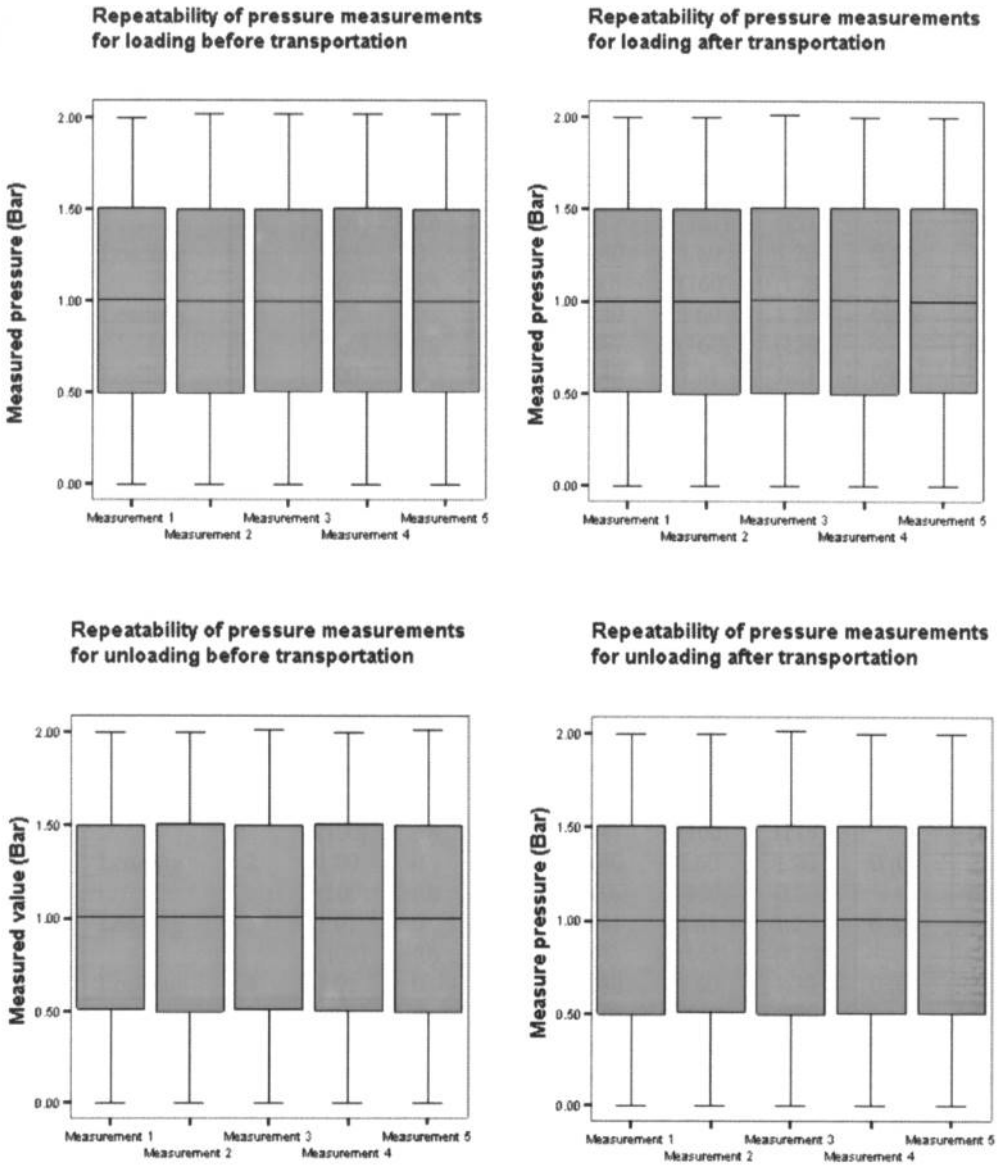


Figure 6-19: Boxplots of the repeatability of five repeated pressure measurements carried out on the same day for the range 0 to 2 Bar (200 kPa).

Before or after transport	Loading or unloading	Measuring session	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentiles 5th	Percentiles 95th	90% Range
Before	Loading	1	1.01 (101)	0 (0)	2.00 (200)	0.40 (40)	1.61 (161)	1.21 (121)	0 (0)	2.00 (200)	2.00 (200)
Before	Loading	2	1.00 (100)	0 (0)	2.02 (2.02)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.02 (202)	2.02 (202)
Before	Loading	3	1.00 (100)	0 (0)	2.02 (2.02)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.02 (202)	2.02 (202)
Before	Loading	4	1.00 (100)	0 (0)	2.02 (2.02)	0.40 (40)	1.61 (161)	1.21 (121)	0 (0)	2.02 (202)	2.02 (202)
Before	Loading	5	1.00 (100)	0 (0)	2.02 (2.02)	0.41 (41)	1.60 (160)	1.19 (119)	0 (0)	2.02 (202)	2.02 (202)
Before	Unloading	1	1.01 (101)	0 (0)	2.00 (200)	0.42 (42)	1.60 (160)	1.18 (118)	0 (0)	2.00 (200)	2.00 (200)
Before	Unloading	2	1.01 (101)	0 (0)	2.00 (200)	0.40 (40)	1.61 (161)	1.19 (119)	0 (0)	2.00 (200)	2.00 (200)
Before	Unloading	3	1.01 (101)	0 (0)	2.01 (201)	0.41 (41)	1.60 (160)	1.19 (119)	0 (0)	2.01 (201)	2.01 (201)
Before	Unloading	4	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
Before	Unloading	5	1.00 (100)	0 (0)	2.01 (201)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.01 (201)	2.01 (201)
After	Loading	1	1.00 (100)	0 (0)	2.00 (200)	0.41 (41)	1.60 (160)	1.19 (119)	0 (0)	2.00 (200)	2.00 (200)
After	Loading	2	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
After	Loading	3	1.01 (101)	0 (0)	2.01 (201)	0.41 (41)	1.61 (161)	1.20 (120)	0 (0)	2.01 (201)	2.01 (201)
After	Loading	4	1.01 (101)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
After	Loading	5	1.00 (100)	0 (0)	2.00 (200)	0.41 (41)	1.60 (160)	1.19 (119)	0 (0)	2.00 (200)	2.00 (200)
After	Unloading	1	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.61 (161)	1.21 (121)	0 (0)	2.00 (200)	2.00 (200)
After	Unloading	2	1.00 (100)	0 (0)	2.00 (200)	0.41 (41)	1.61 (161)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
After	Unloading	3	1.00 (100)	0 (0)	2.01 (201)	0.40 (40)	1.61 (161)	1.21 (121)	0 (0)	2.01 (201)	2.01 (201)
After	Unloading	4	1.01 (101)	0 (0)	2.00 (200)	0.40 (40)	1.61 (161)	1.21 (121)	0 (0)	2.00 (200)	2.00 (200)
After	Unloading	5	1.00 (100)	0 (0)	2.00 (200)	0.41 (41)	1.61 (161)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)

Table 6-5: The summary of between groups repeatability results for all repeated measures taken on the same day. All pressure values are in Bar (kilo-Pascals).

The boxplots in Figure 6-19 show the comparisons between the five-repeated measurement sessions for loading and unloading of the pressure transducer and the effects of transportation and reassembly of the equipment. The x-axis shows the measurements sessions (that is, 1 to 5) and the y-axis shows the measured pressure on a range of 0 to 2 bar (200

kPa). There are no outliers in any of the four graphs. All the boxplots indicate consistency and excellent repeatability between the measurements. There are no between group differences for loading and unloading and for before and after. To explore the between group data shown in Figure 6-19 the median, maximum and minimum values, 5^{th}, 25^{th}, 75^{th} and 95^{th} percentiles and the interquartile and 90% range was calculated for each measurement (see Table 6-5). For all measurements the medians show consistency. Similarly, the minimum and maximum values show consistency with the smallest minimum value of 0 bar (0 kPa) and highest maximum value of 2.02 bar (202 kPa). Each of the percentile groups shows good agreement and repeatability. Overall, the boxplots (see Figure 6-19) and values presented in Table 6-5 indicate excellent repeatability with the loading and unloading response of the transducer and the effects of equipment portability have no significant effect on the repeatability of the pressure measurements.

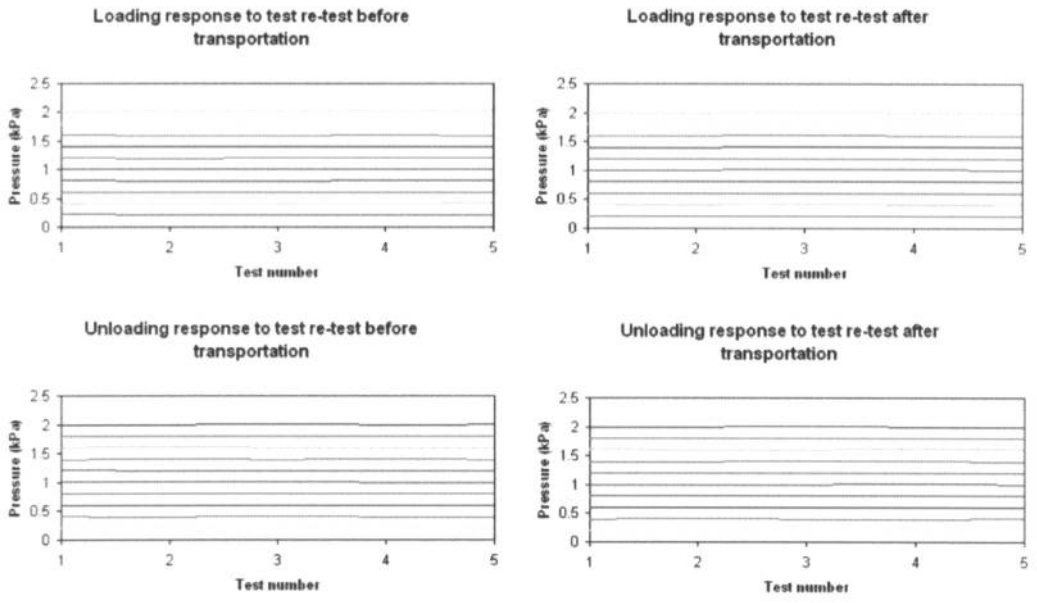


Figure 6-20: Line graphs representing 5 repeated measurements (that is, test 1 to 5) of pressure for loading and unloading, dismantling, transportation and reassembly of the equipment.

To investigate the within group repeatability line graphs were constructed. This allowed for the repeatability of each value that was recorded five times on the tested range of 0 to 2.00 bar (200 kPa) to be compared across the groups (see Figure 6-20). The x-axis shows the 5 measurement sessions

and the y-axis shows the recorded values of pressure in bar. The graphs show the results for loading and unloading, and the effects of moving the equipment. All the graphs show excellent within group repeatability indicated by almost straight lines for each repeated measurement on the test range. The results shown in Figure 6-20 were investigated further by calculating the median, minimum and maximum values, 5th, 25th, 75th and 95th percentiles and interquartile and 90% ranges (see Appendix 5). For each value on the range tested of 0 to 2.00 bar (200 kPa) the medians, minimum and maximum values between all the groups are close suggesting consistency between the within group measurements. The comparison of the percentiles across the group for each respective value on the tested range shows little variability confirming closeness of agreement with measurements. Both the interquartile and 90% ranges are small with the largest interquartile range being 0.01 bar (1 kPa) and 90% range 0.02 bar (2kPa). Overall, the results indicate excellent consistency and repeatability within the groups with no significant differences found.

6.6.1.4 Study 17: Reproducibility of pressure measurements during equipment warm-up

	Coefficient of Variation
BEFORE TRANSPORTATION	
Loading	0.33%
Unloading	0.41%
AFTER TRANSPORTATION	
Loading	0.26%
Unloading	0.30%

Table 6-6: The effects of equipment warm-up on typical errors produced by the pressure system for loading and unloading, before and after dismantling, transportation and reassembly of the equipment. All values are in Bar (kilo-Pascals).

The typical errors caused by the equipment warming-up for loading and unloading and for before and after are shown in Table 6-6. The coefficient of variation values are all small suggesting that measurements taken during equipment warm-up are highly reproducible.

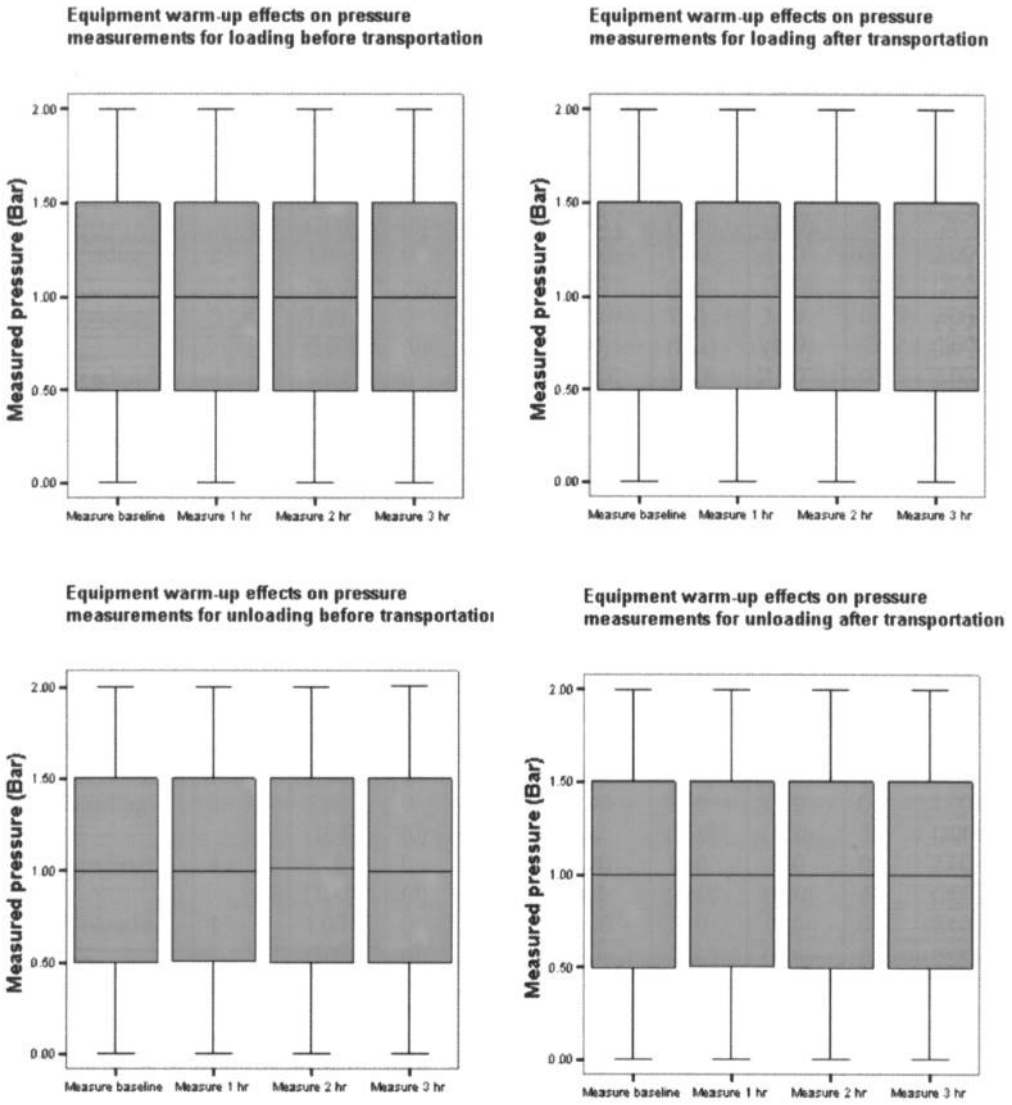


Figure 6-21: Boxplots for loading and unloading, before and after dismantling, transportation and reassembly of equipment for the effects of equipment warm-up on pressure measurements.

Before or after transport	Loading or unloading	Measuring session	Median	Minimum	Maximum	Percentiles		IQR	Percentiles		90% Range
						25th	75th		5th	95th	
Before	Loading	1	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
Before	Loading	2	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
Before	Loading	3	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.61 (161)	1.19 (119)	0 (0)	2.00 (200)	2.00 (200)
Before	Loading	4	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
Before	Unloading	1	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
Before	Unloading	2	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
Before	Unloading	3	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
Before	Unloading	4	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
After	Loading	1	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
After	Loading	2	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
After	Loading	3	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
After	Loading	4	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
After	Unloading	1	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
After	Unloading	2	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
After	Unloading	3	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
After	Unloading	4	1.01 (101)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)

Table 6-7: The summary of between groups reproducibility results for four repeated measurements taken at one-hour intervals on the same day whilst the equipment was warming-up. All pressure values are in Bar (kilo-Pascals).

The boxplots (see Figure 6-21) show the between group comparisons for the effects of warming-up of the equipment on reproducibility of results for loading and unloading of the pressure transducer and the effects of before and after dismantling, transportation and reassembly of the equipment. The x-axis shows the baseline measurements (that is, the pressure measurements on the tested range 0 to 2.00 bar (200 kPa)) taken immediately after switching the equipment on. The other 3 measurements were taken at 1-hour intervals from the baseline recording. The y-axis shows the measured pressure in bar. There are no outliers in any of the

boxplots. All the boxplots show close medians and similar interquartile ranges and whisker distribution (that is, the smallest and largest values that are not outliers). No significant differences exist between the boxplots suggesting that loading or unloading of the pressure transducer or portability of the equipment has little effect on reproducibility of results collected at intervals whilst the equipment was warming up. Further between groups analysis of the data was carried out by calculating the values for the median, minimum and maximum values, the 5th, 25th, 75th and 95th percentiles and the interquartile and 90% range (see Table 6-7). The median, minimum and maximum values show consistency between the groups indicating that equipment warm-up has no significant effect on the reproducibility of pressure measurements. Similarly the percentiles and ranges show similar trends with no differences between values.

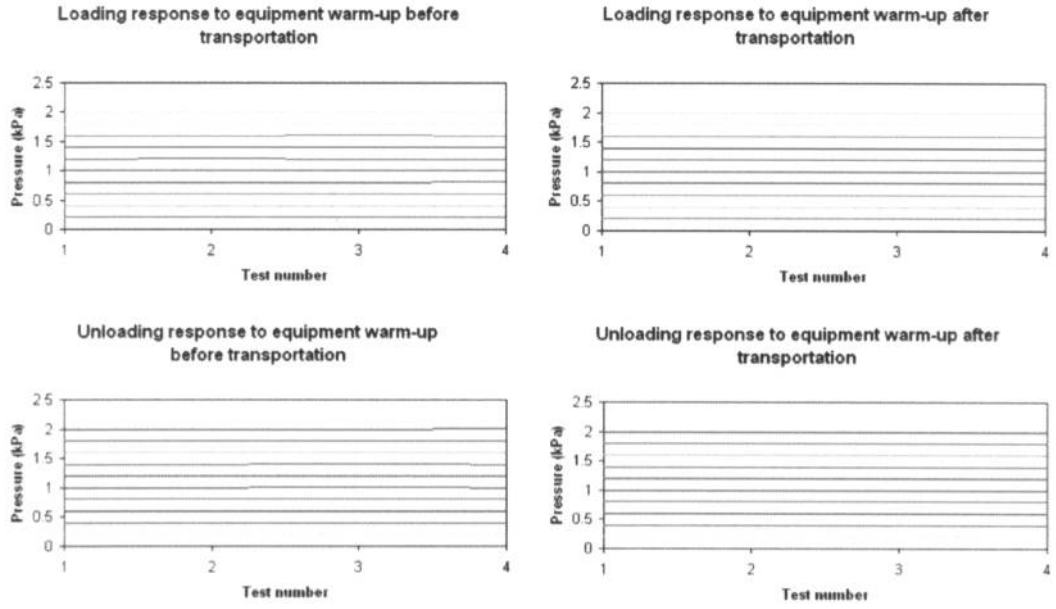


Figure 6-22: Line graphs of loading and unloading response during equipment warm-up for 4 repeated measurements on the scale of 0 to 2 bar of pressure before and after dismantling, transportation and reassembly of the equipment. Test 1 represents the baseline measurement when the equipment was switched on and tests 2 to 5 measurements at 1-hour intervals.

A within groups analysis was carried out by investigating the reproducibility of individual pressure measurements taken during equipment warm-up. The line graph in Figure 6-22 shows the reproducibility of individual measurements taken whilst the equipment was warming up. The x-axis shows the baseline measurements taken immediately after the equipment

was switched on (that is, test number 1) and the 3 measurements taken at 1-hour intervals after baseline. The y-axis shows the measured pressure in KPa. The line graphs show a high level of reproducibility for all the measurements taken with no significant differences between the line graphs for loading or unloading or before and after relocation of equipment. Further analysis of the medians, minimum and maximum values, 5th, 25th, 75th and 95th percentiles and the interquartile and 90% range (see Appendix 5). For the individual cases the medians show closeness to the minimum and maximum values. Similarly the percentiles for the individual cases are similar with the interquartile and 90% ranges showing little variability. This indicates closeness of agreement and excellent reproducibility of the within measurements taken during equipment warm-up.

6.6.1.5 Study 18: Reproducibility of daily pressure measurements

	Coefficient of Variation
BEFORE TRANSPORTATION	
Loading	0.50%
Unloading	0.65%
AFTER TRANSPORTATION	
Loading	0.72%
Unloading	0.41%

Table 6-8: The effects of day to day variation on typical errors produced by the pressure system for loading and unloading, before and after dismantling, transportation and reassembly of the equipment. All values are in Bar (kilo-Pascals).

The coefficient of variation values for measurements taken on different days for loading and unloading of the pressure transducer for before and after transportation are small suggesting pressure measurements taken on different days are highly reproducible (see Table 6-8).

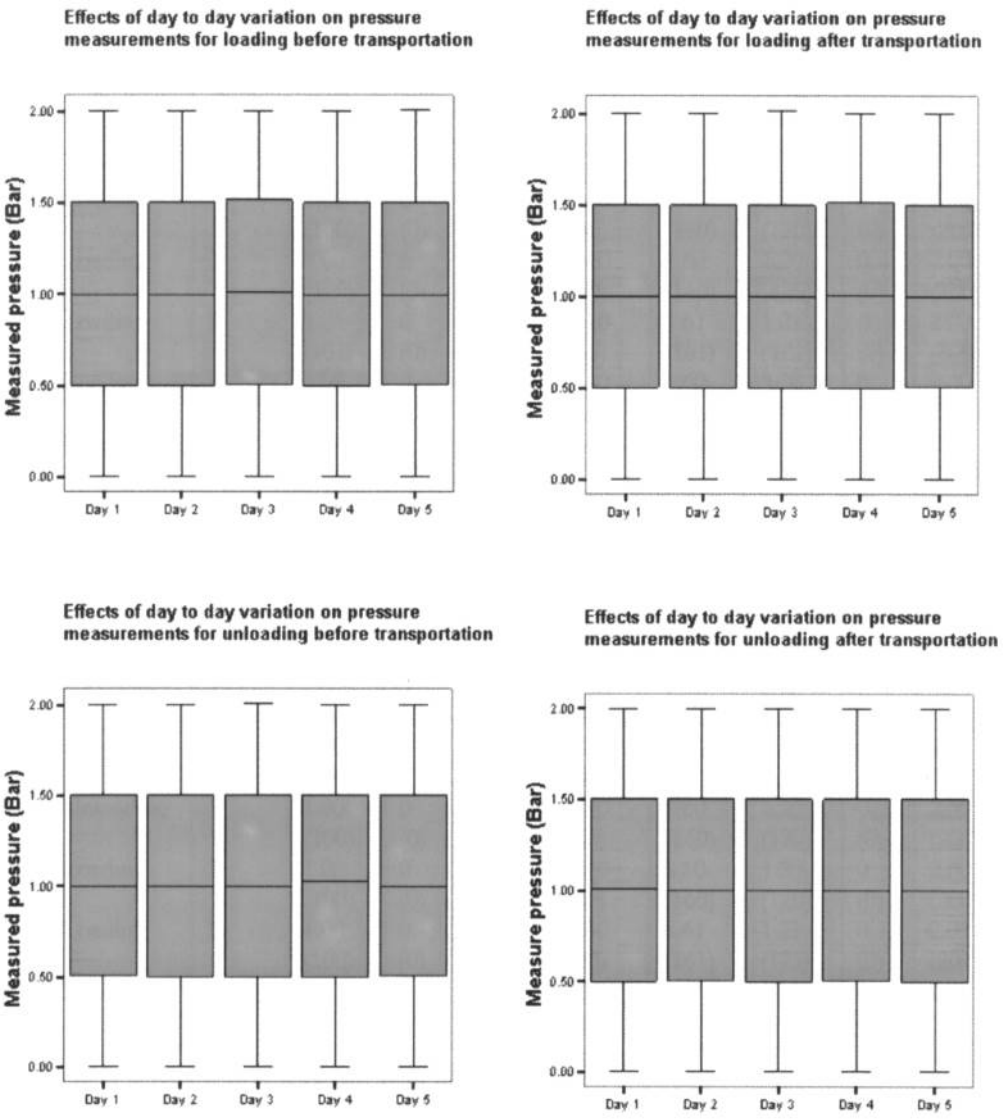


Figure 6-23: Boxplots for loading and unloading, before and after dismantling, transportation and reassembly of equipment for the effects of day-to-day variation on pressure measurements.

Before or after transport	Loading or unloading	Measuring day	Median	Minimum	Maximum	Percentiles		IQR	Percentiles		90% Range
						25th	75th		5th	95th	
Before	Loading	1	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
Before	Loading	2	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
Before	Loading	3	1.01 (101)	0 (0)	2.00 (200)	0.40 (40)	1.61 (161)	1.21 (121)	0 (0)	2.00 (200)	2.00 (200)
Before	Loading	4	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
Before	Loading	5	1.00 (100)	0 (0)	2.01 (201)	0.40 (40)	1.61 (161)	1.21 (121)	0 (0)	2.01 (201)	2.01 (201)
Before	Loading	1	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.61 (161)	1.21 (121)	0 (0)	2.00 (200)	2.00 (200)
Before	Unloading	2	1.00 (100)	0 (0)	2.00 (200)	0.41 (41)	1.60 (160)	1.19 (119)	0 (0)	2.00 (200)	2.00 (200)
Before	Unloading	3	1.00 (100)	0 (0)	2.02 (202)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.02 (202)	2.02 (202)
Before	Unloading	4	1.01 (101)	0 (0)	2.00 (200)	0.40 (40)	1.62 (162)	1.22 (122)	0 (0)	2.00 (200)	2.00 (200)
Before	Unloading	5	1.00 (100)	0 (0)	2.00 (200)	0.41 (41)	1.60 (160)	1.19 (119)	0 (0)	2.00 (200)	2.00 (200)
Before	Unloading	1	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
After	Loading	2	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
After	Loading	3	1.00 (100)	0 (0)	2.01 (201)	0.40 (40)	1.61 (161)	1.21 (121)	0 (0)	2.01 (201)	2.01 (201)
After	Loading	4	1.03 (103)	0 (0)	2.00 (200)	0.40 (40)	1.61 (161)	1.21 (121)	0 (0)	2.00 (200)	2.00 (200)
After	Loading	5	1.00 (100)	0 (0)	2.00 (200)	0.41 (41)	1.60 (160)	1.19 (119)	0 (0)	2.00 (200)	2.00 (200)
After	Loading	1	1.01 (101)	0 (0)	2.00 (200)	0.40 (40)	1.61 (161)	1.21 (121)	0 (0)	2.00 (200)	2.00 (200)
After	Loading	2	1.00 (100)	0 (0)	2.00 (200)	0.41 (41)	1.60 (160)	1.19 (119)	0 (0)	2.00 (200)	2.00 (200)
After	Unloading	3	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
After	Unloading	4	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)
After	Unloading	5	1.00 (100)	0 (0)	2.00 (200)	0.40 (40)	1.60 (160)	1.20 (120)	0 (0)	2.00 (200)	2.00 (200)

Table 6-9: The summary of between groups results for all repeated measures taken on the five different days for the between group analysis. All pressure values are in Bar (kilo-Pascals).

Boxplots were constructed to investigate the between groups variability for loading and unloading and the effects of before and after dismantling, transportation and reassembly of the equipment (see Figure 6-23). The x-axis shows the measurements taken on days 1 to 5 over a test range of 0 to 2.00 bar (200 kPa). The y-axis shows the measured pressure in bar. In

each of the boxplots, measurements taken on days 1 to 5 show significant similarities with small variability between the medians and ranges shown. Furthermore, there are no significant differences between the four graphs suggesting that for pressure measurements taken on different days, loading and unloading and transporting equipment does not significantly affect the reproducibility of measurements. Further analysis of the data presented in the boxplots was carried out by investigating the within groups medians, minimum and maximum values, 5th, 25th, 75th and 95th percentiles and interquartile and 90% ranges (see Appendix 5). The results show excellent between group reproducibility with no significant differences shown between the measurements taken on each set of 5 different days for the medians, minimum and maximum values, 5th, 25th, 75th and 95th percentiles and ranges shown.

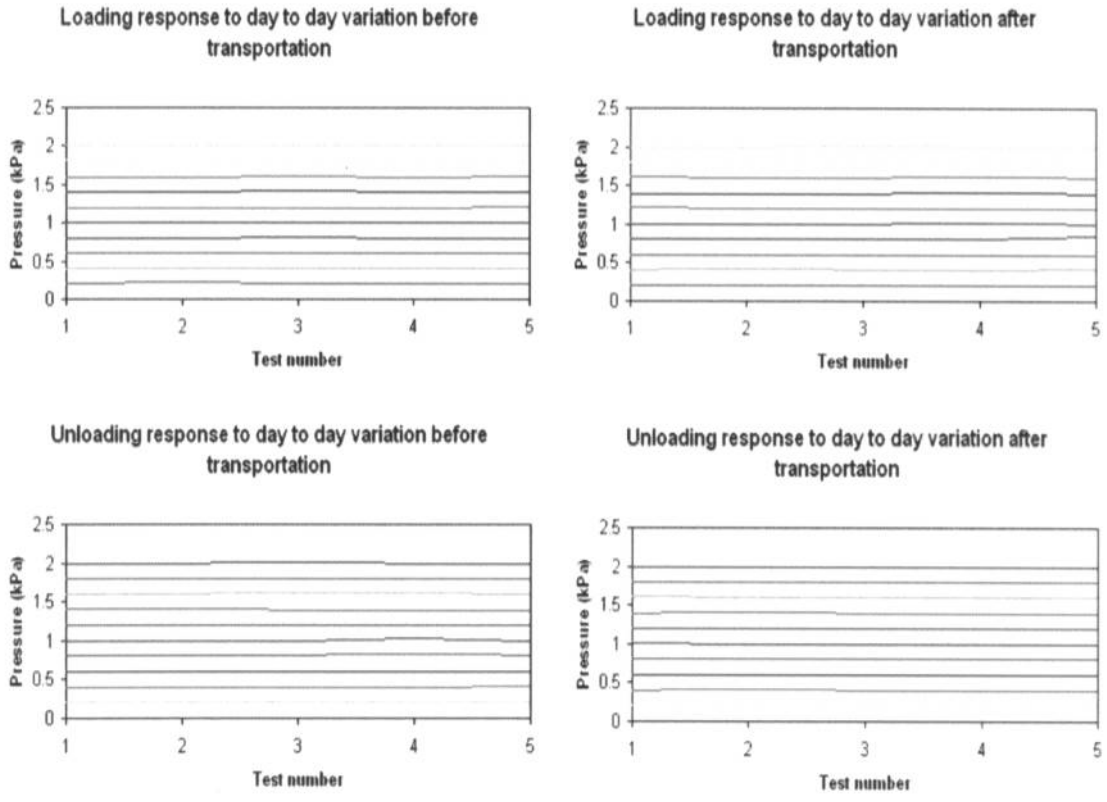


Figure 6-24: Line graphs of loading and unloading response for 5 repeated measurements taken on 5 different days for before and after dismantling, transportation and reassembly of the equipment. Each test was carried out on a scale of 0 to 2 Bar (200 kPa). Test 1 to 5 represents each of the measurement days.

The reproducibility of individual measurements taken on five different days for loading and unloading of the pressure transducer and effects of equipment portability was investigated with line graphs (see Figure 6-24). The results indicate consistency and reproducibility of results taken on different days for all the factors presented with no significant differences between the factors. Further analysis was carried out by calculating the medians, minimum and maximum values, 5^{th}, 25^{th}, 75^{th} and 95^{th} percentiles and the interquartile and 90% ranges (see Appendix 5). The individual medians for the 5 repeated measurements taken on different days show closeness with the minimum and maximum values suggesting small variation of within measurements and a high degree of reproducibility. The percentiles also show a similar trend. The ranges are also small. These findings strengthen the conclusions drawn for the analysis of the line graphs. That is, measurements taken on different days does not affect the reproducibility of individual measurements, nor does loading or unloading the transducer or taking measurements before and after moving the equipment on different days affect the results.

6.6.1.6 Study 19: Hysteresis effects on pressure measurements

The manufacturer of the Novel Pliance-M Expert transducer did not provide us with any hysteresis information regarding their sensor. In this study the tested hysteresis for the Novel transducer was ± 0.0017 bar (0.17 kPa).

6.6.1.7 Study 20: Range, limit of detection and limit of quantification of pressure measurements

The pressure transducer was calibrated and validated for the range of 0 to 2.00 bar (200 kPa). The limit of detection was 0.017 bar (1.7 kPa), which was out with a quantifiable accurate measure for the manometer used, and the limit of quantification was 0.1 Bar (10 kPa).

6.6.2 *In vivo* validation studies of the laser Doppler fluxmeter

The laser Doppler fluxmeter was not revalidated since that was done in section 4.4.2. However, to add to the previous validation studies further *in vivo* studies for linearity, repeatability and reproducibility of day-to-day measurements were conducted.

6.6.2.1 Study 21: *In vivo* repeatability of laser Doppler fluxmeter measurements

The device was designed to apply any quantifiable pressure over any duration of time and in Chapter 5 pressures of 20 kPa, 40 kPa and 80 kPa was applied. In order to test the device over different pressures and time duration the robustness of the system was tested at a higer pressure of 90 kPa over a longer period of time (that is, 5 minutes of pressure application).

The *in vivo* repeatability of the laser Doppler fluxmeter was carried out by taking two repeated measurements of the effects of 90 kPa of plantar foot pressure on skin blood flow. Fourteen healthy participants took part in the study (4 males and 10 females), mean age 35.6 ±13 years and weight 67.6 ±11.6 kilograms. The exclusion criteria and measures to reduce variables that may affect skin blood flow described in Appendix 1 were adopted. Room temperature was kept at 23.0 ±1.0°C. The randomised foot was placed in the measurement shoe device. The subjects then acclimatised in the semi-weight bearing position prior to starting data recording. The measuring protocol consisted of 1 minute baseline recording, followed by the application of 90 kPa of plantar foot pressure that was maintained for 5 minutes, after which the pressure was released and the hyperaemic response measured for a period of 5 minutes. Following a 5-minute recovery break the procedure was repeated a second time.

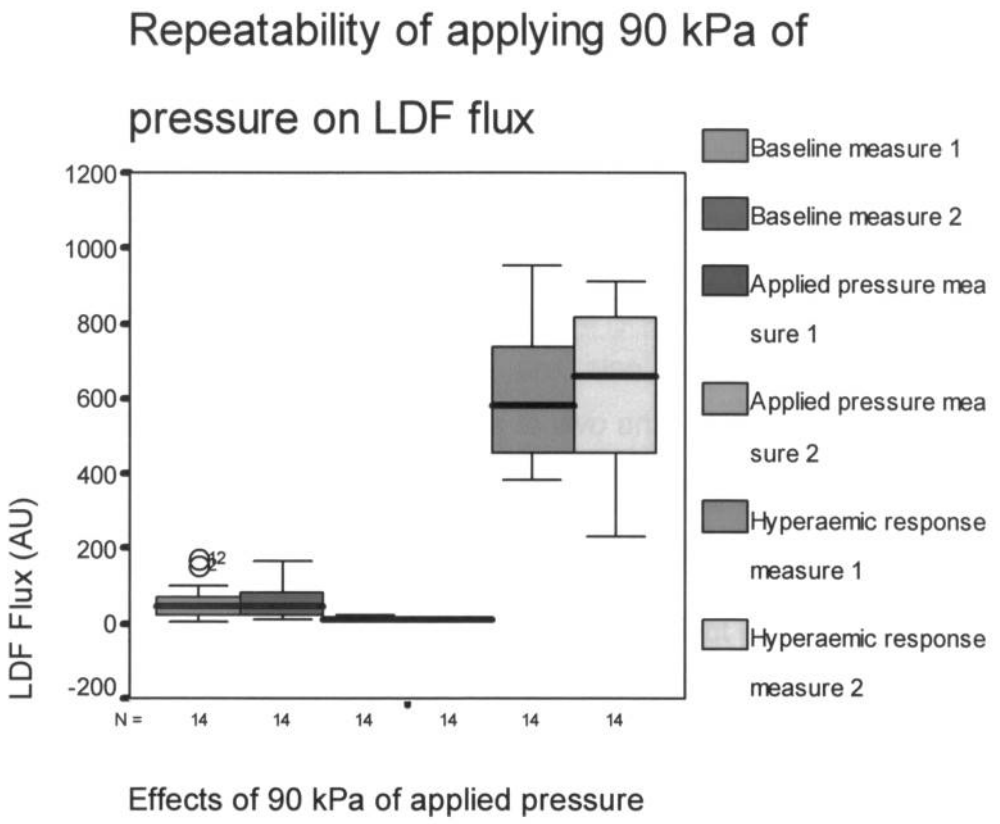


Figure 6-25: Boxplot for the effects of applying 90 kPa of plantar foot pressure on laser Doppler fluxmeter flux for repeated measures (n=14 healthy subjects). Measurements are in perfusion arbitrary units.

	Measure	Median	Minimum	Maximum	Percentiles		IQR	Percentiles		90% range
					25th	75th		5th	95th	
Baseline	1	45.9	3.7	173.5	19.3	77.1	57.8	3.7	173.5	169.8
Baseline	2	43.7	8.2	167.7	23.1	86.2	63.1	8.2	167.7	159.5
Applied pressure	1	9.1	3.4	21.4	5.8	13.0	7.2	3.4	21.4	18
Applied pressure	2	9.1	3.5	18.8	6.4	13.8	7.4	3.5	18.8	15.3
Hyperaemic response	1	579.4	384.5	952.5	444.9	770.2	325.3	384.5	952.5	568
Hyperaemic response	2	657.0	233.0	910.2	442.5	816.7	374.2	233.0	910.2	677.2

Table 6-10: The summary of between groups results for 2 repeated measures taken on the same days for the between group analysis. All laser Doppler fluxmeter flux values are in Arbitrary Units.

The effects of applying 90 kPa of plantar foot pressure on skin blood flow are shown in the boxplots in Figure 6-25 for 2 repeated measurements of laser Doppler flux taken on the same day. The y-axis represents laser Doppler flux in perfusion arbitrary units and the x-axis the number of

measures taken for baseline, during the application of 90 kPa of plantar foot pressure and the post-occlusive hyperaemic response. For the baseline measurements the medians are similar (45.9 and 43.7 AU, see Table 6-10) and the interquartile and 90% ranges also show closeness of agreement (57.8 and 63.1, and 169.8 and 159.5 respectively). There are 2 outliers in the baseline measurement 1 (red boxplot in Figure 6-25) however the maximum values for both measurements are close (173.5 and 167.7 respectively) and the minimum values are also similar (3.7 and 8.2 respectively). Overall, the between groups *in vivo* analysis of baseline laser Doppler flux indicates a good level of repeatability and closeness of agreement.

The results during the application of 90 kPa of plantar foot pressure show a highly significant level of repeatability for the between group analysis as shown in Figure 6-25 and Table 6-10. Both medians are equal at 9.1 arbitrary units and the minimum, maximum and percentiles show closeness of agreement. Finally, the interquartile and 90% range are small suggesting a small distribution around the median and an excellent level of repeatability.

The results for the post-occlusive hyperaemic response show a good level of repeatability although the values are distributed over a wider range than the baseline results or during the application of plantar foot pressure. The median, minimum and maximum values, the percentiles and interquartile and 90% ranges show similar trends. Considering the variability in physiological vascular responses between individuals the results indicate an acceptable level of repeatability.

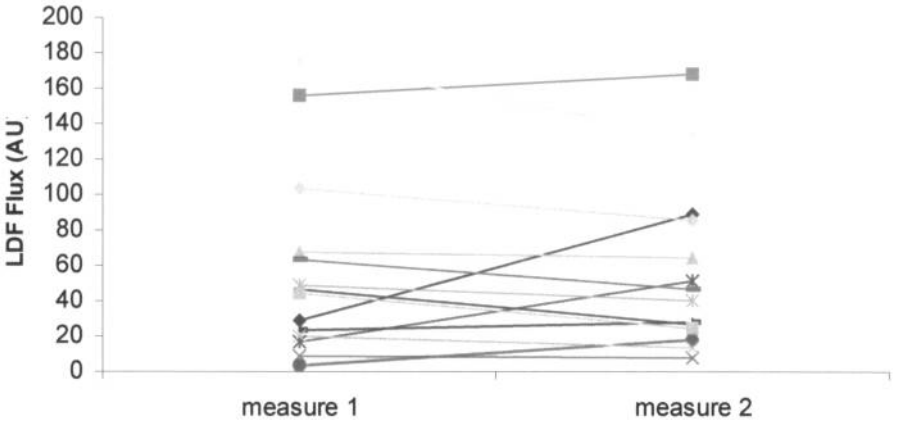


Figure 6-26: Line graphs of 2 repeated measurements taken at baseline on the same day. Laser Doppler fluxmeter flux is measured in Arbitrary Units (AU).

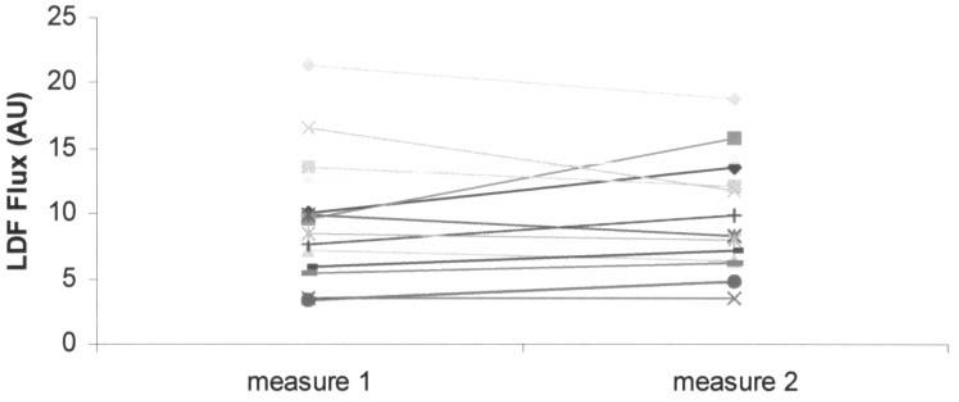


Figure 6-27: Line graphs of 2 repeated measurements taken after 90 kPa of plantar foot pressure had been applied to the skin. All measurements were taken on the same day. Laser Doppler fluxmeter flux is measured in Arbitrary Units (AU).

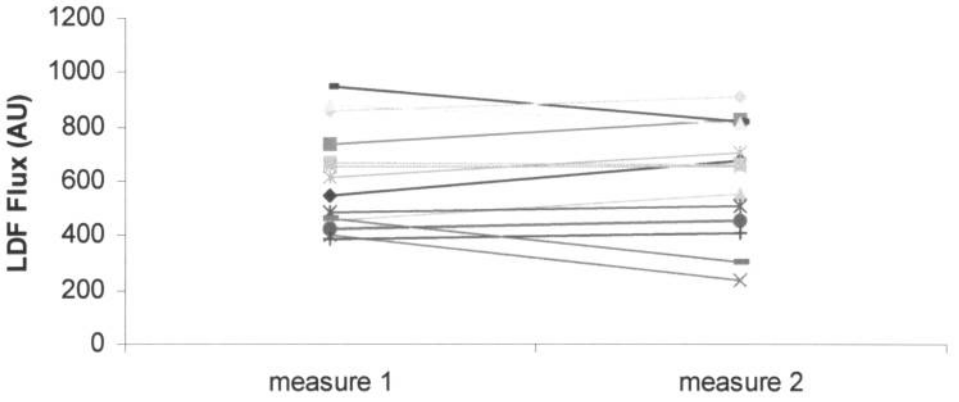


Figure 6-28: Line graphs of 2 repeated measurements taken during the hyperaemic response that occurred when plantar foot pressure was released. All measurements were taken on the same day. Laser Doppler fluxmeter flux is measured in Arbitrary Units (AU).

Line graphs for the within groups repeatability of the 2 measurements taken on the same day are shown in Figure 6-26 (for baseline laser Doppler flux), Figure 6-27 (for the effects on laser Doppler flux during the application of 90 kPa) and Figure 6-28 (for the post-occlusion hyperaemic response). The within groups medians, minimum and maximum values, percentiles and interquartile and 90% ranges are shown in tables in Appendix 6. When plantar foot pressure was applied to the heel the highest level of repeatability was shown, followed by the baseline measurements and the post-occlusive hyperaemic response respectively. The ranges are wide and indicate the inter-individual physiological variability to microcirculatory vascular insults, which are controlled by central and local mechanisms. Overall, the within test re-test measurements show an acceptable level of repeatability within the tested range.

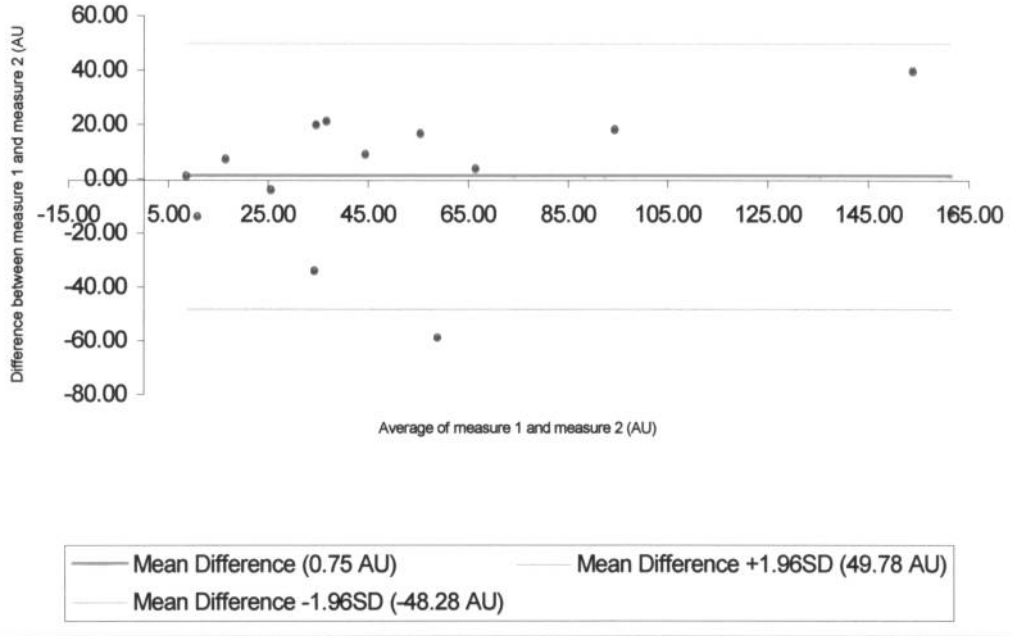


Figure 6-29: Bland/Altman plot for the repeatability of 2 repeated measures for flux taken at baseline on the same day. All measurements are in perfusion arbitrary units (AU). The coefficient of repeatability for baseline is 49.0 AU.

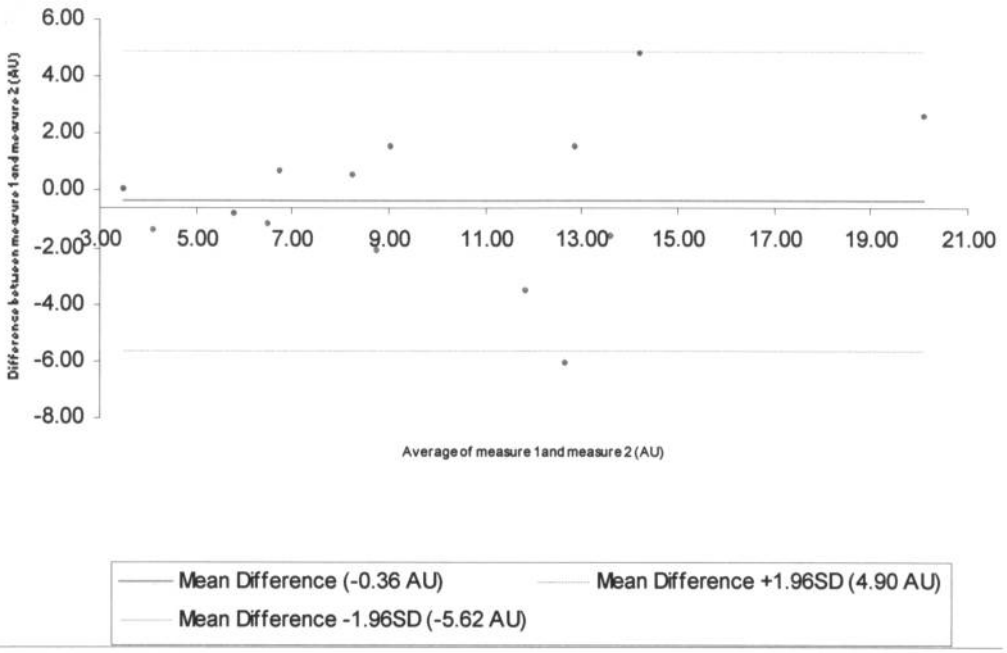


Figure 6-30: Bland/Altman plot for the repeatability of 2 repeated measures for flux taken after the application of 90 kPa of plantar foot pressure on the same day. All measurements are in perfusion arbitrary units (AU). The coefficient of repeatability for the effects of applying 90 kPa of plantar foot pressure on the skin is 5.3 AU.

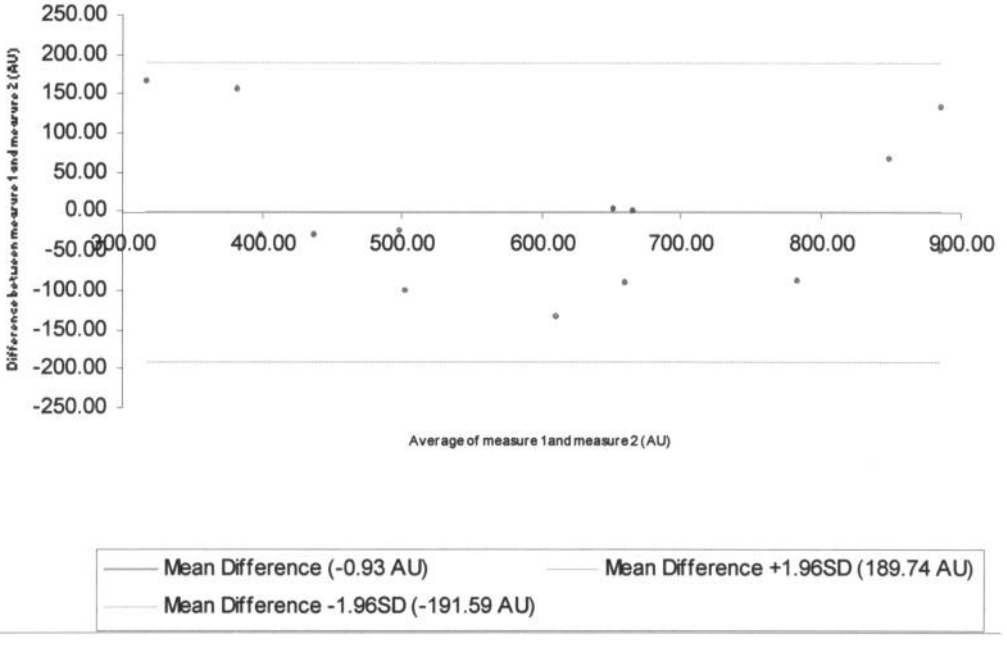


Figure 6-31: Bland/Altman plot for the repeatability of 2 repeated measures for flux taken for the hyperaemic response that occurred after the removal of 90 kPa of plantar foot pressure on the same day. All measurements are in perfusion arbitrary units (AU). The coefficient of repeatability for the hyperaemic response post-occlusion of skin blood flow by plantar foot pressure is 190.7 AU.

Bland/Altman plots were created to calculate the *in vivo* limits of agreement and coefficient of repeatability between the 2 repeated measurements carried out on the same day for laser Doppler flux at baseline, during the

application of plantar foot pressure and the post-occlusive hyperaemic response (shown in different plots from Figure 6-29 to Figure 6-31). The x-axis represents the mean of measure 1 and 2 and the y-axis shows the difference between measure 1 and 2. The "baseline" limits of agreement are 49.78 above and –48.28 Arbitrary Units below the mean difference of 0.75 Arbitrary Units for the study. The coefficient of repeatability for "baseline" measurements was 49.03 Arbitrary Units indicating an acceptable level of repeatability for baseline laser Doppler flux measurements. The measurement of laser Doppler flux "during the application of plantar foot pressure" produced the highest level of repeatability with limits of agreement for the study of 4.90 above and –5.62 Arbitrary Units below the mean difference of –0.36 Arbitrary Units. The coefficient of repeatability for *in vivo* measurements was 5.26 Arbitrary Units indicating a high level of repeatability. Finally, the "post-occlusive hyperaemic response" showed the least level of repeatability with limits of agreement for the test re-test study conducted on the same day of 189.74 above and –191.59 Arbitrary Units below the mean different of –0.93 Arbitrary Units. The coefficient of repeatability for laser Doppler flux was 190.66 Arbitrary Units. Because the "hyperaemic response" occurs on a bigger scale than the previous results for "baseline" and "during the application of pressure" may explain the scalar effects on the coefficient of repeatability.

	Correlation coefficient	***Correlation significance***
FLUX		
Baseline	r = 0.266	P<0.05
During application of pressure	r = -0.260	P<0.05
Hyperaemic response	r = -0.165	P<0.05

Table 6-11: Correlational Coefficent for all the Bland/Altman plots (above) for the *in vivo* repeatability of the laser Doppler fluxmeter measurements.

For the Bland/Altman plots above a correlation coefficient was carried out for baseline, during pressure application and the hyperaemic response to determine any associations between the difference and average of the two flux measurements. A poor correlation was found between the difference and average of the two repeated measurements for flux (see Table 6-11).

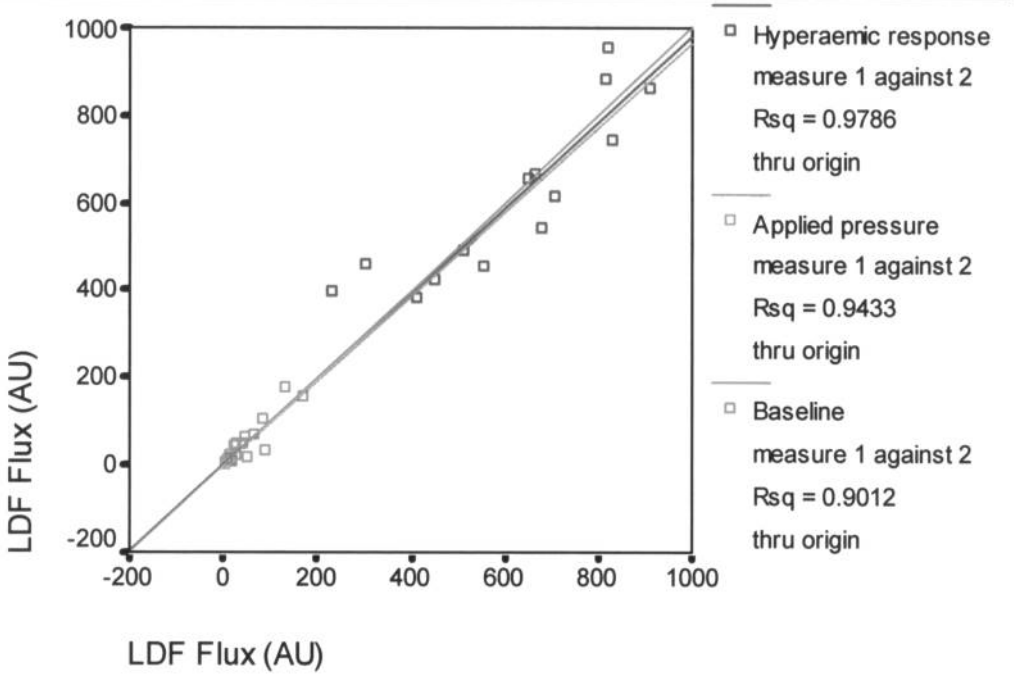


Figure 6-32: Scatterplot with regression lines for the effects of applying 90 kPa of plantar foot pressure on the skin for 2 repeated measurements taken on the same day. Baseline laser Doppler flux and values during the application of 90 kPa of plantar foot pressure on the skin and the hyperaemic response resulting after the removal of the plantar foot pressure are shown.

The linearity of the data presented for the effects of plantar foot pressure on skin blood flow was investigated using a scattergraph between measures 1 and 2 for "baseline" recordings, measurements "during the application of 90 kPa of plantar foot pressure" and the subsequent "post-occlusive hyperaemic response" (see Figure 6-32). The x and y-axis show the laser Doppler flux measurements in Arbitrary Units. The graph indicates a highly significant level of linearity with r-squared correlation coefficient values of 0.98 for "baseline" measurements, 0.94 "during the application of plantar foot pressure" and 0.90 for the "post-occlusive hyperaemic response".

6.6.2.2 Study 22: *In vivo* reproducibility of daily laser Doppler fluxmeter measurements

The reproducibility of day-to-day measurements was recorded by taking two measurements on different days. Ten healthy participants took part in the study (3 males and 7 females), mean age 33.8 ±11.7 years and weight 73.1 ±14.7 kilograms. See Appendix 1 for exclusion criteria and other measures taken. Room temperature was kept on the first day at 23.0 ±0.9ºC and on the second day at 22.7 ±0.62 ºC. On the first day of recordings the subjects

acclimatised in the semi-weight bearing position prior to starting data recording. Following acclimatisation in the semi-weight bearing position subjects were asked to place their randomised foot into the measurement shoe. A baseline measurement of 1 minute was taken followed by the application of 90 kPa of plantar foot pressure for 5 minutes followed by the removal of pressure and the hyperaemic response measured for 5 minutes. The procedure described above was carried out on a different day.

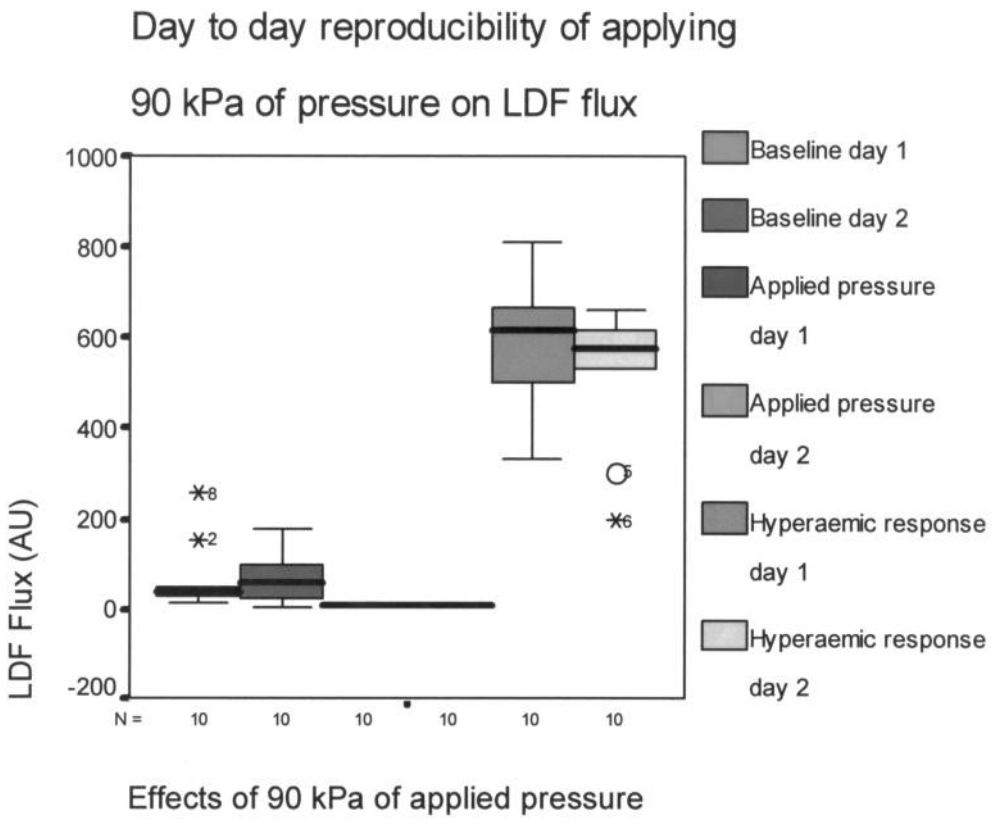


Figure 6-33: Boxplot for the effects of applying 90 kPa of plantar foot pressure on laser Doppler flux for measures taken on 2 different days (n=10 healthy subjects). Measurements are in perfusion arbitrary units.

	Measure	Median	Minimum	Maximum	Percentiles		IQR	Percentiles		90% range
					25^{th}	75^{th}		5^{th}	95^{th}	
Baseline	1	41.5	13.0	257.5	24.7	75.9	51.2	13.0	257.5	244.5
Baseline	2	60.7	5.8	180.1	23.4	98.3	74.9	5.8	180.1	174.3
Applied pressure	1	9.1	4.8	13.6	5.5	12.1	6.6	4.8	13.6	8.8
Applied pressure	2	9.2	3.8	12.4	6.3	11.1	4.8	3.8	12.4	8.6
Hyperaemic response	1	614.9	330.9	810.2	494.7	685.2	190.5	330.9	810.2	479.3
Hyperaemic response	2	577.7	195.9	662.9	474.2	626.4	152.2	195.9	662.9	467

Table 6-12: The summary of between groups results for 2 measures taken on different days for the between group analysis. All laser Doppler fluxmeter flux values are in Arbitrary Units.

The boxplots in Figure 6-33 show the reproducibility of test re-test of 2 measurements taken on 2 different days for effects of applying 90 kPa of plantar foot pressure on skin blood flow. The y-axis represents laser Doppler flux in perfusion arbitrary units and the x-axis the number of measures taken for "baseline", "during the application of 90 kPa of plantar foot pressure" and the "post-occlusive hyperaemic response". The first "baseline" boxplot shows two extreme outliers and thus has a higher maximum value and bigger 90% range than the second "baseline" measurement (see Appendix 6). The outliers may have occurred as a result of variability between subjects. However, excluding these two extreme values the results of "baseline" measurement 1 show less variability than measurement 2 with a smaller interquartile range. Overall, the "baseline" measurements of laser Doppler flux on different days shows a good level of between groups repeatability.

The results "during the application of 90 kPa of plantar foot pressure" have the smallest variability (see Figure 6-33 and Appendix 6). The medians (9.1 and 9.2 Arbitrary Units) and minimum and maximum values are close with very small interquartile ranges (6.6 and 4.8 Arbitrary Units) and 90% ranges (8.8 and 8.6 Arbitrary Units), which are all close to the median. This suggests a highly significant level of repeatability for the between group analysis of laser Doppler flux "during the application of plantar foot pressure".

Finally, the "post-occlusive hyperaemic response" shows the least level of day-to-day reproducibility when compared to the "baseline" and "during plantar foot pressure application" (see Figure 6-33 and Appendix 6). The boxplot shows that the measurements taken on the second day have one outlier and one extreme outlier (again probably due to variability between subjects) and hence the group has the highest maximum value of the two (see Appendix 6). Excluding these two values the measurements taken on the second day show less variability than the measurements taken on the first day. The medians are close (614.9 and 577.7 Arbitrary Units). Taking into account the variability in physiological vascular responses between individuals the results indicate a good level of repeatability for measurements taken on different days.

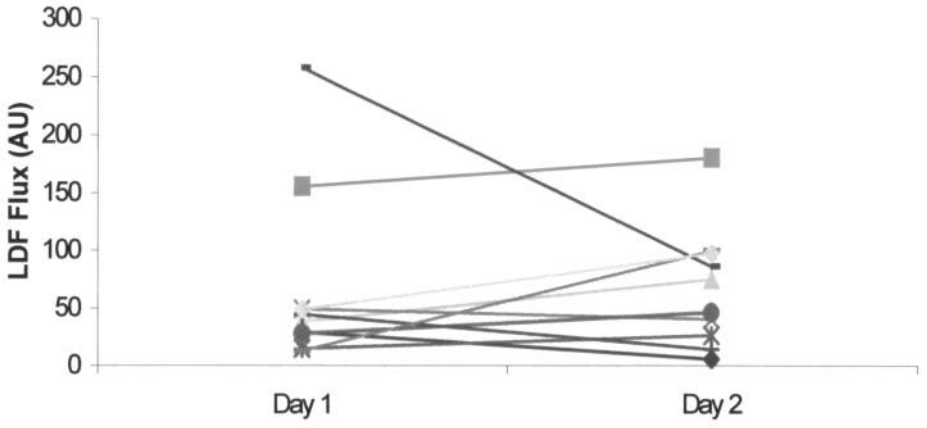


Figure 6-34: Line graphs of repeated measurements of laser Doppler flux taken at baseline on 2 different days. Laser Doppler fluxmeter flux is measured in Arbitrary Units (AU).

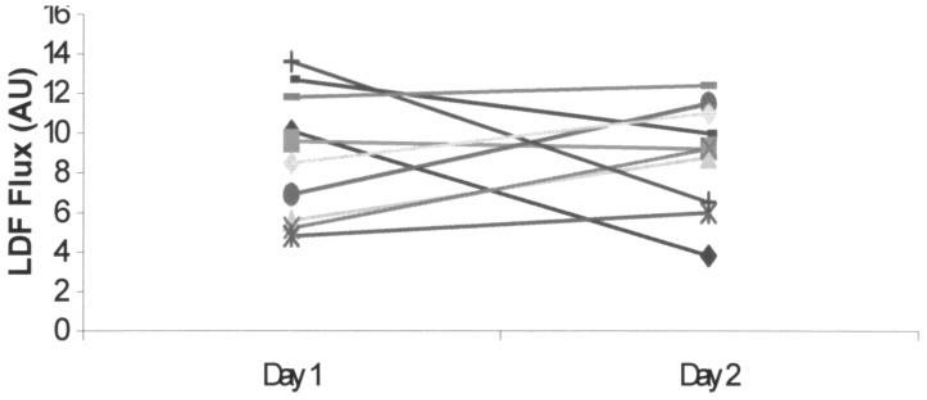


Figure 6-35: Line graphs of repeated measurements of laser Doppler flux taken on 2 different days after 90 kPa of plantar foot pressure had been applied to the skin. Laser Doppler fluxmeter flux is measured in Arbitrary Units (AU).

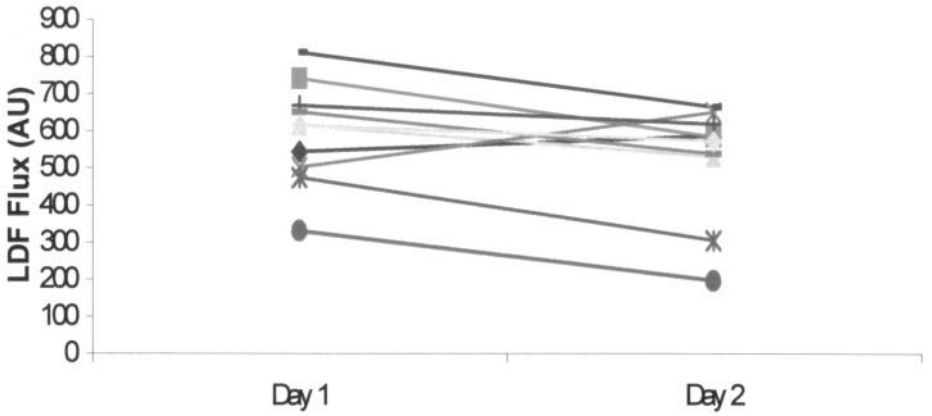


Figure 6-36: Line graphs of repeated measurements of laser Doppler flux taken on 2 different days during the hyperaemic response that occurred when plantar foot pressure was released. All measurements were taken on the same day. Laser Doppler fluxmeter flux is measured in Arbitrary Units (AU).

The within groups reproducibility of measurements taken on different days was investigated using line graphs (see Figure 6-34 to Figure 6-36) and analysis of medians, minimum and maximum values, percentiles and interquartile and 90% ranges are shown in Appendix 6. The x-axis shows the days the measurements were taken and the y-axis the laser Doppler flux in Arbitrary Units. Figure 6-34 shows the results for "baseline" laser Doppler flux and 2 extreme outliers can be seen in day 1. For "baseline" measurements, excluding the two outliers in day 1, the within group repeatability is good with acceptable interquartile and 90% ranges (see Appendix 6). Figure 6-35 shows the effects on laser Doppler flux "during the application of 90 kPa of plantar foot pressure" with excellent levels of repeatability as can be seen in Appendix 6 with closeness of agreement between the medians, minimum and maximum values, percentiles and interquartile and 90% ranges. Figure 6-36 shows the "post-occlusion hyperaemic response" and a trend to a small reduction in measured values on day 2 observed. The ranges are wider than the data presented for "baseline" and "during occlusion of skin blood flow", however considering the inter-individual physiological variability to microcirculatory vascular insults, the overall within measurements taken on different days show a good level of repeatability within the tested range.

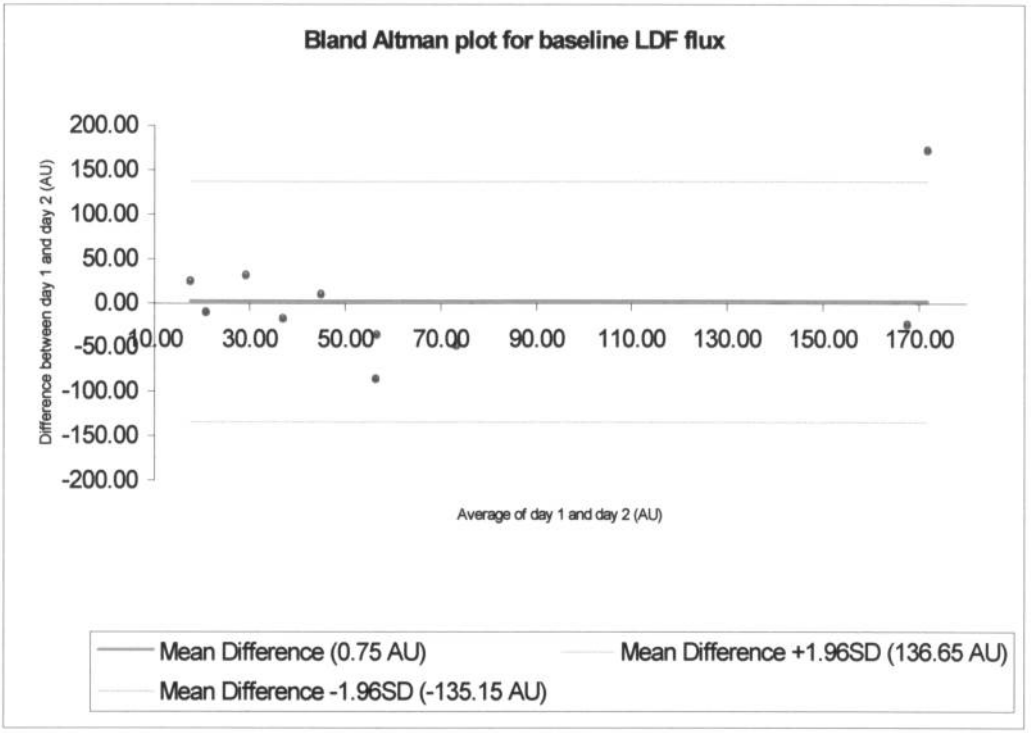


Figure 6-37: Bland/Altman plot for 2 repeated measures of laser Doppler flux taken at baseline on the 2 different days. All measurements are in perfusion arbitrary units (AU). The coefficient of repeatability for baseline is 135.9 AU.

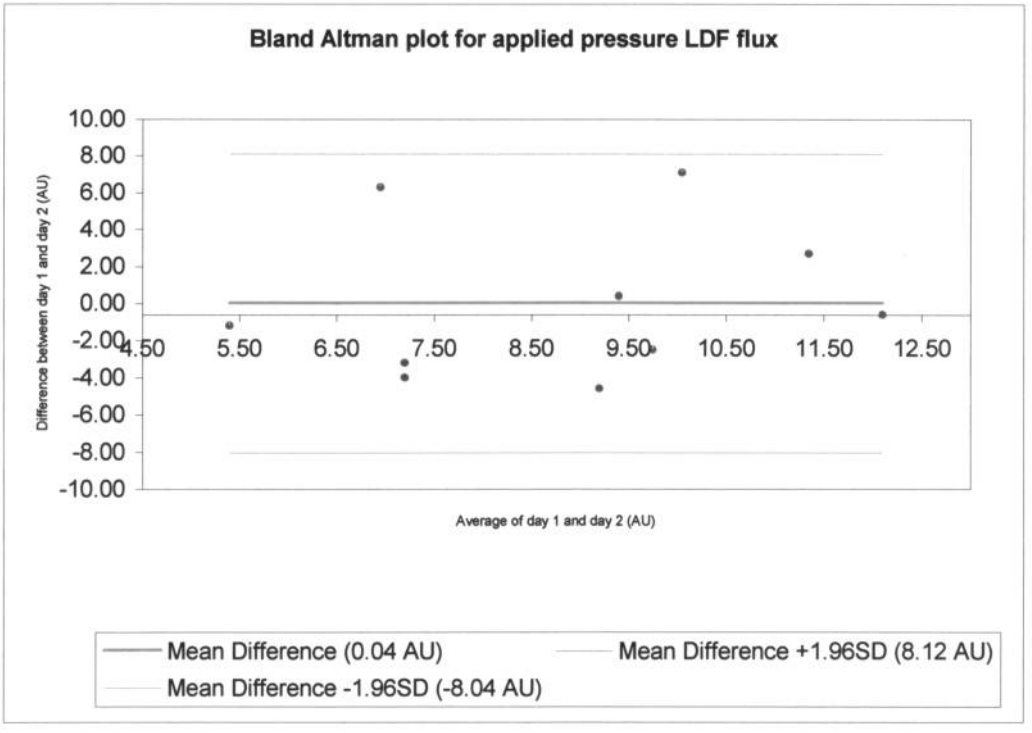


Figure 6-38: Bland/Altman plot for the repeatability of 2 repeated measures for flux taken after the application of 90 kPa of plantar foot pressure on the same day. All measurements are in perfusion arbitrary units (AU). The coefficient of repeatability for the effects of applying 90 kPa of plantar foot pressure on the skin is 8.08 AU.

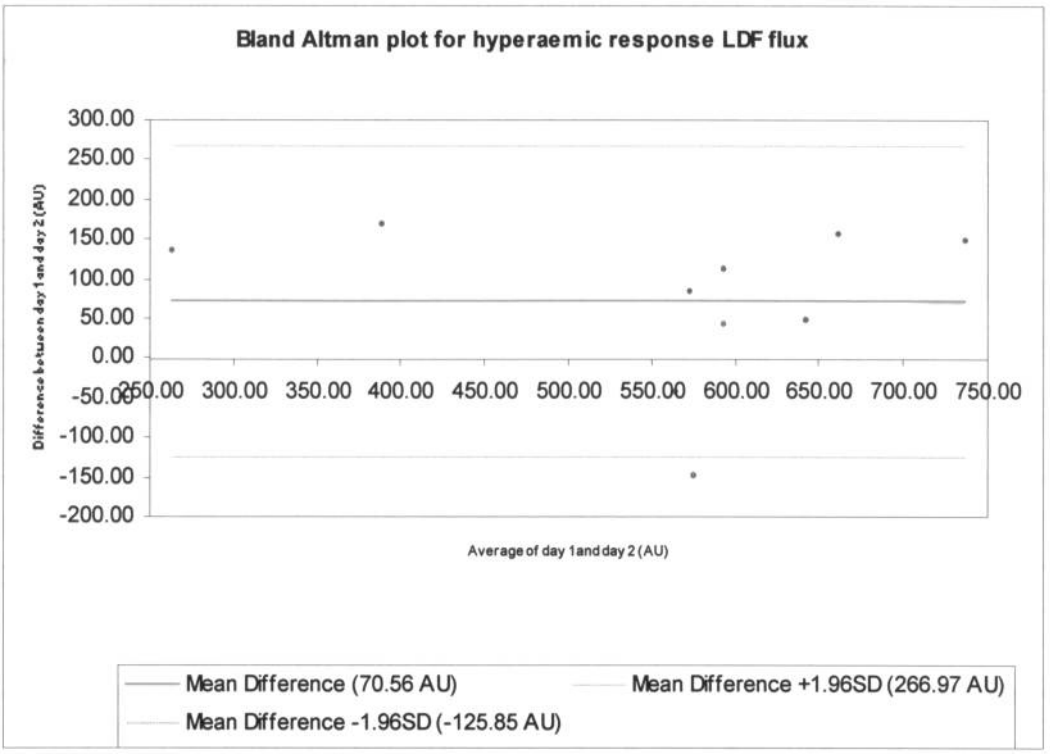


Figure 6-39: Bland/Altman plot for the repeatability of 2 repeated measures for flux taken for the hyperaemic response that occurred after the removal of 90 kPa of plantar foot pressure on the same day. All measurements are in perfusion arbitrary units (AU). The coefficient of repeatability for the hyperaemic response post-occlusion of skin blood flow by plantar foot pressure is 196.41 AU.

The limits of agreement were studied using Bland/Altman plots and the coefficient of repeatability between measurements taken on two different days calculated and are shown in Figure 6-37 to Figure 6-39 for laser Doppler flux at "baseline", "during the application of plantar foot pressure" and the "post-occlusive hyperaemic response" respectively. The x-axis represents the mean of measure 1 and 2 and the y-axis shows the difference between measure 1 and 2. For the *in vivo* reproducibility study carried out the "baseline" limits of agreement were 136.65 above and –135.15 Arbitrary Units below the mean difference of 0.75 Arbitrary Units. The coefficient of repeatability for "baseline" measurements was 135.90 Arbitrary Units and is high in relation to the value presented for repeatability described in the previous section. This high figure may have been influenced by the extreme values in day 2 measurements.

"During the application of 90 kPa of plantar foot pressure" limits of agreement for the study were 8.12 above and –8.04 Arbitrary Units below the mean difference of –0.04 Arbitrary Units with a coefficient of repeatability of 8.08 Arbitrary Units indicating a high level of repeatability.

Finally, a similar trend to the results in the previous section 6.6.2.1 was shown with the "post-occlusive hyperaemic response" showing the least level of reproducibility. The limits of agreement for the reproducibility of measurements taken on different days were 266.97 above and –125.85 below the mean different of 70.56 Arbitrary Units. The coefficient of repeatability for laser Doppler flux was 196.41 Arbitrary Units. Overall, the results indicate an acceptable level of reproducibility for measurements taken on different days.

	Correlation coefficients	***Correlation significance***
FLUX		
Baseline	r = -0.261	P<0.05
During application of pressure	r = 0.304	P<0.05
Hyperaemic response	r = 0.067	P<0.05

Table 6-13: Correlation coefficients for all the Bland/Altman plots (above) for the *in vivo* reproducibility of daily measurements using the laser Doppler fluxmeter.

From Table 6-13 the correlation coefficeints for all the Bland/Altman plots above was carried out for baseline, during pressure application and the hyperaemic response to determine any associations between the difference of repeated measurements carried out over two days and average of the two flux measurements taken on different days. Poor correlation coefficients were found between the difference and average of the two measurements taken on different days for flux (see Table 6-13).

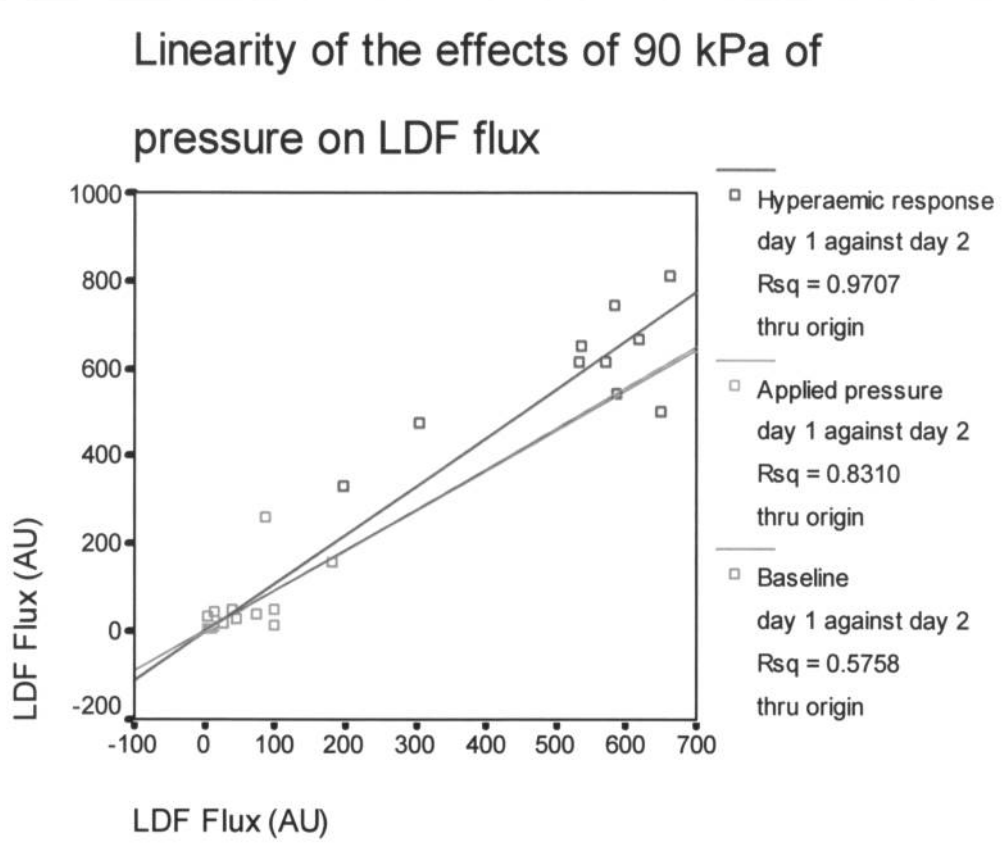


Figure 6-40: Scatterplot with regression lines for the effects of applying 90 kPa of plantar foot pressure on the skin for measurements taken on 2 different days. Baseline laser Doppler flux and values during the application of 90 kPa of plantar foot pressure on the skin and the hyperaemic response resulting after the removal of the plantar foot pressure are shown.

A scatterplot was produced to investigate the effects on linearity of taking *in vivo* laser Doppler flux measurements on different days. The results for laser Doppler flux measurements taken "during the application of plantar foot pressure" on the tissues and during the "post-occlusive hyperaemic response" produced a highly significant correlation between measurements taken on different days with r-squared values of 0.83 and 0.97 respectively. The "baseline" measurements did not produce a significant correlation with an r-squared value of 0.58.

6.7 Discussion

A number of recommendations were made in Chapter 4 (section 4.7) regarding improvements to the developed system. First it was suggested that a method to electronically synchronise pressure and skin blood flow should be developed. Measuring the effects of in-shoe plantar foot pressure on skin blood flow involves adapting two completely different devices and making them work as one. The laser Doppler fluxmeter (DRT4)

communicates directly with a computer and measures skin blood flow. It may be connected to an AN02 box to enable it to receive and send analogue signals. However the limitation of this system is that it does not have a synchronisation switch. Using the iontophoresis function of the laser Doppler fluxmeter DRT4, an O/P1 signal output of 2.49 volts can be obtained via the AN02 box. This signal was used to trigger the developed midway synchronization box. Once the synchronization box was triggered it transmitted a pulsed signal of 5 volts to operate the Novel Pedar Standard system. Each of the pulsed signals recorded 1 pressure frame. The synchronization box is stopped by a second 2.49 volts impulse emitted by the DRT4 via the AN02 box, which in turn stops the Novel pressure system. The described method of using a synchronization box may also be used to synchronise other systems and open new areas of research. Other modifications made were the reduction of the piston surface area to the same size as the sampling area of the laser Doppler fluxmeter transducer.

In Chapter 4 (section 4.2.3) the diameter of the piston was the same size as the strain gauge, which was larger than the diameter of the skin blood flow transducer. Hence, a limitation of the previous system was that the pressure area sampled was larger than the area sampled by the skin blood flow transducer. In this chapter modifications were made and the piston surface area was reduced to exactly the same size as the skin blood flow transducer.

The validation showed that the Novel Pedar Standard pressure system to be an excellent mobile piece of equipment to measure plantar foot pressure. The system was tested over a range 0 to 2 bar (200 kPa) as pressures used to study the effects of plantar foot pressure on skin blood flow in patients with rheumatoid arthritis would not exceed this range. The capacitive pressure transducer produced an excellent level of linearity against applied loads. The level of accuracy was high with a coefficient of repeatability of 0.011 bar (1.1 kPa) or 0.55% of full-scale deflection. The results were highly repeatable and reproducible for measurements taken during equipment warm-up and on different days. Hysteresis is a problem

that is likely to affect pressure systems since the properties of materials used to construct the pressure transducer (that is, compressibility and material memory problems) may produce different results depending on whether the value obtained was recorded when the equipment was loading or unloading. For example, in running the transducer materials may compress quickly to provide a loaded output but on unloading the materials memory may not allow the transducer to react as quick as the unloading pressure, hence the result obtained by loading and unloading for the same applied pressure may vary. Since the intended purpose of the developed device was to be used for static recording hysteresis is unlikely to influence the results significantly. However, the pressure system was tested for hysteresis and the results showed that the hysteresis effects were not significant for the proposed study. Overall, the pressure system is suitable for the proposed study in rheumatoid arthritis and appears not to be significantly affected by loading or unloading or by the effects of dismantling, transportation and reassembly of the equipment.

When measuring skin blood flow *in vivo* the physiological vascular responses are controlled centrally by the autonomic nervous system and locally by metabolic substances (Cragg, 2000[a]; Cragg, 2000[b]; Johnson, 1978). Inter-individual physiological variability means that although the skin blood flow measuring equipment *in vitro* produces excellent results, *in vivo* vascular responses vary between individuals hence the repeatability and reproducibility will be more variable than *in vitro* studies where experimental factors are better controlled (discussed in Chapter 4, section 4.5). Various authors have reported not only intra-individual spatial variability but also a significantly larger variability for resting (baseline) laser Doppler flux (Van Den Brande *et al.,* 1993; Yamaguchi *et al.,* 1991; Gush *et al.,* 1984; Sundberg, 1984). In Chapter 4 (section 4.7) recommendations were made with regards to carrying out more *in vivo* equipment validation studies for the laser Doppler fluxmeter. Two *in vivo* studies were carried out to investigate the repeatability and reproducibility (on different days) of skin blood flow measurements recorded during the study protocol involving taking a "baseline" measurement, followed by a measurement "during the

application of plantar foot pressure" and finally, measuring the "post-occlusive hyperaemic" response after the plantar foot pressure was removed. The first *in vivo* validation study investigated the repeatability of skin blood flow measurements taken before, during and after 90 kPa of plantar foot pressure was applied to the skin. The measurements taken during the application of plantar foot pressure were most repeatable followed by baseline measurements and the post-occlusive hyperaemic response produced the greatest variability. Gush *et al.* (1984) reported the repeatability of laser Doppler fluxmeter measurements taken on the same site to be within 10% and differences between adjacent sites greater than 10%. Other studies have found that repeated measurements on one site but turning the probe by 90 degrees before each measurement affected the repeatability of results (Ingólfsson *et al.,* 1994). Thus, since the validation *in vivo* repeatability study involved repositioning of the foot between the test re-test measurements it may be affected by intra-site variability since the error margin for reposition of the foot in the shoe device is likely to be greater than the intra-site variation that occurred in Ingólfsson *et al.'s* (1994) study which only involved turning the probe by 90 degrees on the same site. For the baseline measurements the baseline coefficient of repeatability was excellent at 49.0 Arbitrary Units, which over the tested range of 0 to 952.5 Arbitrary Units (that is, the maximum value recorded for the post-occlusive hyperaemic response) was 5%. Reports of the repeatability of the reduction in laser Doppler flux following the application of plantar foot pressure or the hyperaemic response have not been reported previously. The coefficient of repeatability for test re-test during the application of 90 kPa of plantar foot pressure was 5.26 Arbitrary Units or 1% of full -scale deflection for the tested range, that is, results are highly repeatable. Finally, the post-occlusive hyperaemic response produced a coefficient of repeatability of 190.66 Arbitrary Units or 20% of full-scale deflection, which may be acceptable for the following study since the measurements of the hyperaemic response occur over a higher scale of values.

The second laser Doppler fluxmeter *in vivo* equipment validation study investigated the reproducibility of taking measurements on different days.

The reproducibility of laser Doppler flux measurements recorded on different days varied with some authors reporting some day-to-day variation but other not finding such variations (Bircher *et al.,* 1994; Yamaguchi *et al.,* 1991; Sundberg, 1984; Tenland *et al.,* 1983). The validation study reported the reproducibility of laser Doppler flux measurements taken on different days as 135.90 Arbitrary Units (that is, coefficient of repeatability) or 17% of full-scale deflection for the range tested of 0 to 810.2 Arbitrary Units and showed greater variability between measurements taken on different days. The reproducibility of laser Doppler flux measurements taken on different days was close to repeated measurements taken on the same day with a coefficient of repeatability value of 8.08 Arbitrary Units or 1% of full-scale deflection. The reproducibility of laser Doppler flux for measurements taken on different days was close to measurements taken in the previous *in vivo* validation repeatability experiment with a coefficient of repeatability of 196.41 Arbitrary Units or 24% of full-scale deflection. The *in vivo* validation study suggests that the laser Doppler fluxmeter when used in conjunction with the Novel pressure system produces a higher variability when used on different days for "baseline" and "post-occlusive hyperaemic response" measurements but not for flux measurements "during loading".

6.8 Conclusion

The device to measure the effect of quantifiable plantar foot pressure on skin blood flow was improved by substituting the strain gauge pressure system with the Novel pressure system. In addition, further *in vivo* validation of the laser Doppler fluxmeter DRT4 system was carried out. The device was tested to be suitable to measure the effects of plantar foot pressure on skin blood flow.

6.9 Limitations and recommendations

The developed synchronisation box allowed for the first time to electronically synchronise the laser Doppler fluxmeter (DRT4) and the Novel Pedar Standard. Such system will allow for future studies into the effects of external pressure on skin blood flow with minimal bioengineering equipment development. The principle of a synchronisation box, successfully used in

the mentioned study, may be adapted to synchronise other systems. The following systems were synchronised using the synchronization box allowing for future studies into biomechanics and/or skin blood flow:

Pedar mobile system (in-shoe pressure system)
GaitRite system (gait analysis system)
Isotrak system (electromagnetic goniometry system)
Laser Doppler fluxmeter DRT4 (skin blood flow system)

CHAPTER 7

A STUDY INVESTIGATING THE EFFECTS OF PLANTAR FOOT PRESSURE ON SKIN BLOOD FLOW IN PATIENTS WITH RHEUMATOID ARTHRITIS

CHAPTER 7: A Study investigating the effects of plantar foot pressure on skin blood flow in patients with rheumatoid arthritis

7.1 Introduction

Rheumatoid arthritis usually affects the feet, causing pain on weight bearing that can severely restrict ambulation and is a major cause of disability (Hodge *et al.,* 1999; Calabro, 1962). In addition the skin is often dry, devitalised and thin. Further complications results from vasculitis, which is the most serious extra-articular manifestation that causes inflammation of small and/or large blood vessels. The vasculitis may lead to vascular infarcts, ischaemic ulcers and gangrene. In some cases peripheral neuropathy secondary to vasculitis may occur. In such cases the loss of pain sensation and increased plantar foot pressure caused by the deformity and atrophy of the fat pad may lead to neuropathic ulceration. However, this affects few and neuropathic ulceration in rheumatoid arthritis is not very common.

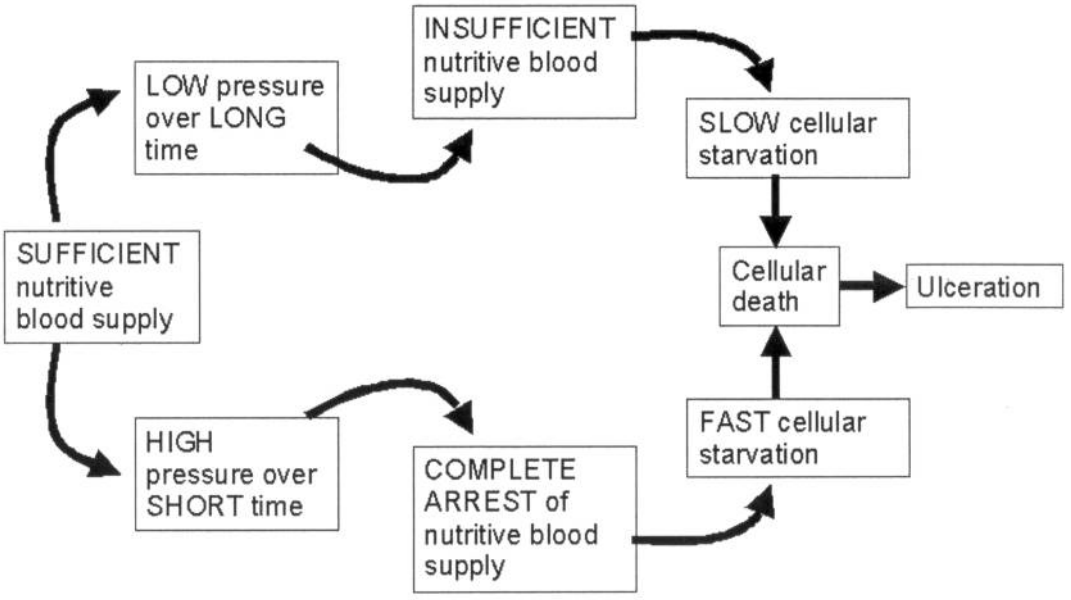


Figure 0-1: The relationship between the application of external pressure to the skin and it effect on the microcirculation leading to cellular death and soft tissue breakdown.

The overall picture is a patient with a high-risk foot with a combination of factors that may result in soft tissue breakdown. It is well documented that soft tissue breakdown may result from the application of low pressure over a

long period of time or high pressure over a short period of time (see Figure 0-1). No studies have investigated the effects of plantar foot pressure on skin blood flow in patients with rheumatoid arthritis. The majority of studies investigating this in 'normals' and other conditions have carried out their investigations with subjects in a supine position and only a few studies have been carried out with subjects in a semi-weight bearing or walking position. This chapter aims to contribute and strengthen current knowledge with regards to the effects of plantar foot pressure on skin blood flow in patients with rheumatoid arthritis.

7.2 Epidemiology

7.2.1 Incidence

The average incidence of rheumatoid arthritis worldwide is approximately 1.4 to 1.9 cases per 1000 per year (Hochberg *et al.,* 1990; Hochberg, 1981). In the United Kingdom the average five-year incidence rate of rheumatoid arthritis is 6% (Hochberg, 1981).

Bukhari *et al.* (1998) investigated the baseline characteristics of 194 patients who showed radiographical signs of joint erosion in their feet only or their hands only or both. The incidence of the joint erosion one to two years after disease onset in the feet only was 20.1% (n=39) compared to 41.2% (n=80) of hand involvement only or 38.7% (n=75) with erosion in both hands and feet (Bukhari *et al.,* 1998).

7.2.2 Prevalence

The prevalence of rheumatoid arthritis in the Caucasian population is approximately 1%, however it is appears to be higher in rural West Germans (5%) and the Finish population (3%) (Saag *et al.,* 1996; Hochberg *et al.,* 1990; Hochberg, 1981). In rheumatoid arthritis the prevalence is the highest worldwide in two North American Indian populations, the Chippewa Indians with 6.8% and the Yakima Indians with 3.4% (Hochberg, 1981). In the Chinese populations the prevalence appears to be approximately 0.3%, one of the lowest worldwide (Lau *et al.,* 1996). With regards to prevalence, differences appear to exist between rural and urban areas. In African

countries differences in prevalence seems to exist between rural (0.1%) against urban (0.9%) populations (Hochberg, 1981). Similar findings have been reported in Taiwan with the lowest cases in rural areas (260 per 100,000[7]) followed by suburban areas (780 per 100,000[7]) and urban areas (930 per 100,000[7]) (Lau *et al.,* 1996). Thus, rheumatoid arthritis appears to be more prevalent in the American Indian and Caucasian populations and less prevalent in the Chinese and African populations. In addition the disease seems to be more prevalent in urban than rural areas.

The prevalence of foot involvement in rheumatoid arthritis is very high. Amongst ninety-nine patients with clinically proven rheumatoid arthritis 94% had foot and ankle involvement at least once since diagnosis. Ankle problems had developed in 42%, heel pain in 1%, midfoot pain in 5%, forefoot problems in 28%, both forefoot and ankle problems in 14% and the rest complained of ankle, midfoot and forefoot involvement (Michelson *et al.,* 1994). Other studies (mentioned earlier) have reported foot involvement on rheumatoid arthritis in 16% of newly diagnosed cases and in 80 to 100% of patients with long-term disease (Klenerman, 1995; Geppart *et al.,* 1992; Jacobs, 1984). This suggests that foot problems in rheumatoid arthritis are very common and increase with disease duration.

7.3 Age and gender

The prevalence of rheumatoid arthritis in females is approximately 2 to 3 times greater than males (Lau *et al.,* 1996; Hochberg, 1981). In both men and women the prevalence of rheumatoid arthritis increases with increasing age, affecting 2% of males and 5% of females over the age of fifty-five (Symmons *et al.,* 1994; Hochberg, 1981). In the United Kingdom Turner *et al.* (2001) also found the prevalence of rheumatoid arthritis to increase with age and affect more females than males with an average prevalence for males of 1.6% and females 2.6% over the age of 45 years (Turner *el at.,* 2001). The Norfolk Arthritis Register study found the incidence of rheumatoid arthritis in men to be 0.14 per 1000 and 0.36 per 100 for women (Symmons *et al.,* 1994).

[7] Figures adjusted for age and gender.

Bukhari *et al.* (1998) found the mean age at onset was significantly less for the group with radiographical evidence of foot joint erosions only (49 years) when compared to the group with hand involvement only (66 years) or the group with involvement of joints in both the hands and feet (59 years). In addition the study found a higher percentage of males in the group with foot involvement only (39.5%) compared to hand involvement only (30%) or both (29.3%). Bukhari *et al.* (1998) suggested that the higher proportion of younger males with joint erosions in their feet first might be accounted by mechanical factors such as occupation and the finding that they had been prescribed fewer steroids at baseline.

7.4 Effects of rheumatoid arthritis on plantar foot pressure

Patients with long-term disease duration have reported worse pain than patients with short-term disease duration (Coster *et al.,* 2001). Other studies have reported that foot involvement in rheumatoid arthritis is 16% at diagnosis and 90 to 100% for those with a ten-year history (Geppart *et al.,* 1992; Jacobs, 1984). In a review Klenerman (1995) reported significant foot pathology in 80-91% of patients with long standing disease, with both forefoot and rearfoot involvement.

rheumatoid arthritis causes forefoot deformity and increases plantar foot pressure (described in page 24) resulting in the development of adventitious bursae (especially over the metatarsal heads) that can become inflamed and lead to painful ambulation (Resnick *et al.,* 1999; Wiener-Ogilvie, 1999; Braid, 1996). In addition, degeneration of the fat pad also occurs. Resnick *et al.* (1999) compared the fatty acid composition of the heel's fat pad in patients with rheumatoid arthritis (n=11) and non-rheumatoid controls (n=8). The heels pads of patients with rheumatoid arthritis had a higher concentration of saturated fatty acids and decreased concentrations of unsaturated fatty acids (Resnick *et al.,* 1999). Such differences may have resulted from differences in enzyme activity between the two groups (Jacobsson *et al.,* 1990). Saturated fatty acids increase the viscosity of fat reducing the ability of the heel to absorb and dissipate energy produced

during human locomotory activity. The increased stress may cause degeneration of the heel's septal system and may accelerate atrophy of the heel's fat pad (Resnick *et al.,* 1999). Thus, in rheumatoid arthritis atrophy of soft tissue structures makes weight-bearing bony surfaces more prominent and increases plantar foot pressure.

Young *et al.* (1995) found that in rheumatoid patients with metatarsalgia the plantar soft tissue depth was thinner than controls. Hodge *et al.* (1999) found a correlation between "average" plantar foot pressure and pain (although there are also other factors involved in producing pain sensation) but not between "peak" plantar foot pressure and pain suggesting that in the rheumatoid foot pain appears to be unrelated to peak plantar foot pressure. Furthermore, the velocity of gait and stride length has been correlated with levels of pain with increased pain correlated with reduced gait velocity and stride length (Platto *et al.,* 1991). Gait velocity, cadence and stride length is reduced in patients with rheumatoid arthritis with a late heel rise, but is more marked in those with rearfoot valgus deformity (Keenan *et al.,* 1991).

7.5 Effects of rheumatoid arthritis on blood flow

Vasculitis is the most serious extra-articular manifestation of rheumatoid arthritis and can affect both small and large vessels, although it mainly affects patients with seropositive IgM and IgG rheumatoid factor erosive disease (Harper, 2002; Snowden *et al.,* 1995; Geppert *et al.,* 1992). Thus, patients affected by vasculitis tend to have a high titre of IgM rheumatoid factor or a high titre of other isotopes for rheumatoid factor (Harper, 2002; Snowden *et al.,* 1995). During active vasculitis C-reactive proteins and the erythrocyte sedimentation rate are also raised (Harper, 2002). From the current research evidence it appears that vasculitis is mediated by B cell overactivity, immune complex formation and complement consumption (Snowden *et al.,* 1995).

Clinically evident vasculitis is present in approximately 2 to 5% of patients, although the subclinical form is more common (Harper, 2002). Watts *et al.* (2000) reported the incidence of primary systemic vasculitis in Norfolk (UK) to be 19.8 per million and increased with age. Muscle biopsy has shown

lymphocytic vasculitis affecting 10% of seropositive patients (Snowden *et al.,* 1995). In patients with rheumatoid arthritis vasculitis may present as a bland obliterating endarteritis or an inflammatory focal and segmented vasculitis (Geppert *et al.,* 1992). The most common cutaneous manifestation of rheumatoid vasculitis is as purpura (Harper, 2002). In active disease the obliterating vasculitis form affects digital arteries and small brown/black spots of infarcted soft tissue are common on the nail folds and apices of digits (Braid, 1996; Snowden *et al.,* 1995; Geppert *et al.,* 1992). Edwards (1980) carried out a study to investigate whether a relationship exists between external pressure and digital vasculitis in patients with rheumatoid arthritis. Ninety-two rheumatoid nailfold vascular infarcts were compared with sites of blanching during a pinch and power grip. The study reported that such lesions are not randomly distributed and appear to occur at sites of pressure related blanching with 86% (n=79) of infarcts occurring in the blanched/pressure sites. In addition, the sites of vasculitis were noted to occur at the edge of areas of blanching. Thus, the possibility exists that at the edge of the pressure blanching areas the small vessels containing static blood may facilitate vascular damage and lead to the vascular infarcts. Another interesting observation was that no vasculitic nailfold infarcts were found in subjects whose digits were so severely affected that they had lost the ability to form a grip involving the terminal phalanges (Edwards, 1980).

In the inflammatory focal and segmented vasculitis major vessels may be involved presenting a clinical picture of infarctions, cutaneous ulceration, peripheral gangrene, mononeuritis multiplex and in some cases death (Geppert *et al.,* 1992). Major vasculitis appears to mainly affect the small and medium sized arteries supplying the skin and extremities and the vasa-nervosum (Snowden *et al.,* 1995).

At sites where the peripheral vascular supply is diminished ischaemic ulcers may develop (Braid, 1996). Neuropathic ulcers may also develop as a result of peripheral sensory neuropathy or sensorimotor neuropathy secondary to vasculitis. Nerve damage occurs as a result of the vasculitic disease

disturbing the blood supply to nerves leading to nerve damage (Geppert *et al.,* 1992). Peripheral sensory neuropathy is common in 40% of patients with rheumatoid vasculitis (Harper, 2002). Where ulceration exists healing may be delayed as a consequence of the presence of vasculitic disease (Geppert *et al.,* 1992).

7.6 Skin involvement in rheumatoid arthritis

In patients with rheumatoid arthritis the skin is often dry, devitalised and thinner than healthy skin due to the disease and secondary to medication (for example, the use of corticosteroids) and inactivity (Braid, 1996; Geppert *et al.,* 1992). In such patients the slightest graze may result in soft tissue breakdown and necrosis (Braid, 1996). Furthermore, the effects of plantar foot pressure on the atrophied skin may lead to painful callosities or ulceration (Geppert *et al.,* 1992). However, where painful callosities exist podiatric intervention with debridement only reduces pain for approximately seven days but the plantar foot pressure distribution is not significantly altered (Woodburn *et al.,* 2000). However, the F-Scan used by Woodburn *et al.* (2000) averages pressures and differences in plantar foot pressure distribution may have been noted if peak pressures had been looked at instead. Where soft tissue breakdown occurs the risk of infection is increased as a result of a disordered immune response, neuropathy and ischaemia (Braid, 1996; Geppert *et al.,* 1992).

Endothelium-dependant and independent skin vessels reactivity has been shown to diminish with age and may explain both structural and functional skin changes with aging (Algotsson *et al.,* 1995). In rheumatoid arthritis skin blood flow vasodilatory responses in patients appears to be diminished. Ferrell *et al.* (2001) carried out a study using the laser Doppler imager and iontophoresis to determine the skin's microcirculatory reactivity to acetylcholine in eight patients classified using the ARA criteria as suffering from rheumatoid arthritis. The study concluded that patients with rheumatoid arthritis, especially those with acute flares, show evidence of marked impairment of endothelium-dependant dilation (Ferrell *et al.,* 2001). With regards to the effects of external pressure on skin blood flow, these findings

suggest that when patients with rheumatoid arthritis and healthy controls are compared the post-pressure hyperaemic response may differ if the two groups exhibit different levels of vasodilatory responses. Furthermore, endothelium-dependant vasodilatation impairment has been associated with cardiovasvascular risk factors (Filer *et al.* 2001). Thus, patients with rheumatoid arthritis have an increased risk of cardiovascular disease with increased diastolic blood pressure, elevated thrombotic variables and high mortality rate (McEntegart *et al.,* 2001). The elevated cardiovascular factors may suggest that such patients also have an increased risk of microcirculatory disease as found by Ferrell *et al.* (2001).

7.7 Method

7.7.1 Testing protocol

The same testing protocol was used for the control and rheumatoid arthritis groups. All rheumatoid arthritis subjects complied with the American Rheumatism Association (ARA) criteria (see Appendix 7). Room temperature and humidity was monitored and the date and subject identity number for each measuring session was recorded (see Appendix 8). The modified developed device was assembled as described in section 6.3. A measuring protocol was set up using the iontophoresis software for the laser Doppler flowmeter as described in section 5.5.8. The measuring protocol consisted of a 1-minute baseline recording, followed by the application of quantifiable plantar foot pressure for the duration of 5 minutes, followed by the removal of plantar foot pressure and the recording of the hyperaemic response for 5 minutes (that is, the measuring protocol lasted a total of 11 minutes for each applied plantar foot pressure). On the Novel Pliance-M Expert recording software (that is, the pressure system) the type of synchronisation was set to "on each picture". Three pressures were selected (80, 90 and 100 kilo-Pascals) because some of the rheumatoid arthritis subjects tested were at high risk. Thus, it was decided not to apply pressures above 100 kPa since these could harm the soft tissues or cause pain. The sequence that plantar foot pressure was applied to the skin was randomised to remove any bias arising from applying the same repeated sequence (for example, 80 kPa, 90 kPa and 100 kPa). A

measuring session at each respective plantar foot pressure was carried out with a recovery period of five minutes between the sessions.

Subjects complying with the study's specific inclusion and exclusion criteria and the general exclusion criteria (see Appendix 1) were invited to participate in the study. In addition, measures to reduce factors that may affect skin blood flow were also adopted (see Appendix 1). One foot was selected at random for all the measurements. Skin blood flow was measured in the centre of the heel and at the control site over the 3rd metatarsal head.

Prior to commencing the recording session the synchronisation box was switched onto the standby mode. The measuring shoe's piston was checked to be in the unloaded position. The procedure for synchronised data recording described in section 5.5.8 was followed. During the periods of application and removal of the plantar foot pressure the electronic marker switch was pressed to record this period where plantar foot pressure was being applied or removed. The plantar foot pressure and the skin blood flow files were then saved for future analysis. The saved files could then be statistically analysed using their individual respective programs or together when merged into SPSS or Microsoft Excel for further analysis. The recorded files were then closed and new files opened ready for the next recording session.

7.7.2 Rheumatoid arthritis subjects

Ethical approval was sought from Borders Research Ethics Committee (NHS Borders). All subjects were recruited from the Rheumatology Department, Borders General Hospital, NHS Borders, over a period of three months (from 21st June 2002 to 19th September 2002). Potential subjects were identified by the consultant, using the patient questionnaires and an information leaflet (see Appendix 9 and 10), handed out prior to their appointment. Patients who were interested in participating in the study were screened for suitability using the data capture sheet (see Appendix 12). The data capture sheet recorded information regarding the subject's age, gender, weight, height, shoe size, drug history, pain in the past 4 weeks,

duration of Rheumatoid disease, Rheumatoid Factor (see Appendix 7) and erythrocyte sedimentation rate (see Appendix 7) results. In addition, the sheet was used to screen the subject for the inclusion/exclusion criteria and accept those suitable. Those subjects who were suitable were invited to participate in the study. The researcher then explained the purpose of the study and what was required of the subject. Any questions asked by patients were answered and a study number allocated to each participant after recording their demographic details. The demographic details where held separate to the data capture sheets in a secure locked cabinet to comply with the data protection act. Finally, subjects were asked to complete the consent form (see Appendix 13) and it was made clear that they could withdraw from the study at any time and that participating or withdrawing from the study would not affect the standards of care that they were receiving. At the end of the study all the patients' demographic details were destroyed and only the anonymous data kept.

INCLUSION CRITERIA:

Diagnosed with rheumatoid arthritis following the ARA criteria (see Appendix 7)

EXCLUSION CRITERIA:

Current foot and/or leg ulcer(s)

Current foot and/or leg gangrene

Diagnosed with Raynaud's phenomenon

7.7.3 Control subjects

Ethical approval was sought from Borders Research Ethics Committee (NHS Borders) and from the Ethics Research Committee (Queen Margaret University College). All subjects were recruited from the Borders General Hospital NHS Trust, Queen Margaret University College and Lothian Council staff over a 3-month period (from 24th July 2002 to 30th September 2002). Healthy subjects were recruited using the study recruitment poster and study information sheets (see Appendix 11) that were posted on notice

boards at the above named locations and also e-mailed. Those subjects who responded to the invitation were screened using the data capture sheet for healthy controls (see Appendix 12). The data capture sheet recorded the participant's age, gender, height, weight, drug history, shoe size and inclusion/exclusion criteria. Those healthy subjects that satisfied the study's specific criteria and the general exclusion criteria (see Appendix 1) were invited to participate. For those who accepted to take part the researcher explained the purpose of the study and what was required of the subject. Any questions asked by the subjects were answered and a study number was given to each subject prior to completion of the consent form (see Appendix 13). To comply with the data protection act the procedure detailed in section 7.7.2 was followed.

INCLUSION CRITERIA:

No medical history of rheumatoid arthritis

EXCLUSION CRITERIA:

See section 7.7.2.

7.7.4 Statistical analysis

7.7.4.1 Analysis between the rheumatoid and control group

Following the report from the Standardisation Group of the European Society of Contact Dermatitis a guideline was produced for measurement of cutaneous blood flow using laser Doppler flowmetry (Bircher *et al.,* 1994). The guidelines recommended that for analysis the results can either be presented as the values directly read from the display or as the difference from biological zero because the precise origin of the biological zero signal may be multifactorial and is still a matter for debate (see section 2.1.4 Biological zero). Hence, some authors may argue that since the precise origin of biological zero is not known the readings from the display should be presented whilst others believe that although its precise origin is not known it is still an artifarct reading and thus the difference from biological zero should be worked out. In this study data was referenced to biological zero.

A number of authors have interpreted the hyperaemic response following arterial occlusion and was previously described in Chapter 5. Analysing the effects of pressure on skin blood flow in the heel using spline curves and errors bars has previously been reported (Mayrovitz *et al.,* 1997; Mayrovitz *et al.,* 1998). The data was analysed at 30 seconds intervals for each of the pressures applied (that is, 80 kPa, 90 kPa or 100kPa) using error bars with a line bisecting all the means at each interval. The error bars represent the mean and 95% confidence intervals at each 30 seconds period. This allowed for a visual comparison of the curves for the variables compared.

More in-depth non-parametric statistical analysis was carried out at each interval for the variables compared and probability values produced. The Mann-Whitney U test is a non-parametric test used as a distribution free analogue and as an alternative to the independent samples t-test. It assesses whether two populations have the same location (Field, 2002; Kinnear *et al.,* 2000). The data was non-parametric and hence a Mann-Whitney U test was selected for comparisons between both groups at each 30 seconds interval.

7.7.4.2 Analysis of the within subjects effects

An extensive literature search revealed no articles that have analysed the within subjects effects by comparing the values of the baseline, occluded and post-occlusion periods following the application of pressure on the skin. However, previous authors have described the various parts of the laser Doppler fluxmeter curve that occurs before, during and after arterial occlusion and was discussed earlier in Chapter 5. In light of this, within subject analysis for each of the groups at applied pressures of 80 kPa, 90 kPa and 100 kPa was carried out. The data were non-parametric and Friedman tests were carried out. The Friedman test is a non-parametric test used to compare observations repeated on the same subjects (Kinnear *et al.,* 2000).

7.8 Results

7.8.1 Control probe

No statistical differences were found for both between and within group analysis of the data (see APPENDIX 13: Control probe analysis). For all recordings, both for the control and rheumatoid arthritis groups, the control probe showed a stable trace with no deviation from baseline suggesting that any changes in skin blood flow that occurred at the heel were related to the application of plantar foot pressure.

7.8.2 Cohort characteristics of rheumatoid arthritis subjects

Twelve patients with rheumatoid arthritis volunteered for the study; however one was excluded since the patient also suffered from diabetes mellitus. Patients with diabetes mellitus are known to suffer from both micro and macro vascular disease hence it was decided to exclude this patient since any disturbances found in the micro-vascular response to applied plantar foot pressure could be to do with the diabetes mellitus and not rheumatoid arthritis or a combination of both conditions. Of the eleven patients that participated in the study 1 was male and 10 were females. The mean age of the cohort with rheumatoid arthritis was 51 years (Standard Deviation of 12) with a range of 30 years and minimum and maximum values of 35 to 65 respectively. The group had a mean shoe size of 6 (Standard Deviation of 2.0) with a range of 7 and a minimum value of 4 and maximum value of 11 (see Table 7-3).

The cohort had an average mass of 75 Kilograms (Standard Deviation of 23) and a range of 69 Kilograms. The minimum and maximum mass values were 51 and 120 kilograms respectively. The mean height was 1.66 metres (Standard Deviation of 0.10) with a range of 0.39 and a minimum value of 1.53 and maximum value of 1.92 (see Table 7-3).

The body mass index is used to classify obesity and is calculated by dividing the weight in Kilograms by the height in metres square (that is, body mass index = Kg/m^2). The calculated number is then used to categorise individuals into a scale of underweight to extremely obese (see

Table 7-1). The rheumatoid arthritis group had a body mass index of 27.0 (Standard Deviation of 6.7) and a range of 23.1 with a minimum and maximum values of 20.0 and 43.1. Thus, the cohort has an average distribution of overweight individuals ranging from normal weight to extremely obese (see Table 7-3).

Less than 18.5	Underweight
18.5 to 24.9	Normal
25.0 to 29.9	Overweight
30.0 to 34.9	Obese
35.0 to 39.9	Very obese
40.0 or greater	Extremely obese

Table 7-1: The categories for the body mass index.

All patients met the ARA rheumatoid arthritis criteria for the diagnosis of rheumatoid arthritis (see Appendix 7). Morning stiffness was present in 64% (n=7) of the subjects. All subjects had arthritis in the hands and arthritis of 3 or more joints. 91% (n=10) suffered from symmetrical arthritis with only 9% (n=1) suffering from asymmetrical rheumatoid arthritis. Rheumatoid nodules were present in 64% (n=7) of patients and 36% (n=4) had no presence of nodules. Radiological evidence of arthritic joint changes was present in all patients with 18% (n=2) showing changes in the feet only, another 18% (n=2) in the hands only and 64% (n=7) in both feet and hands. In addition, 45% (n=5) had joint involvement in areas other than the feet and hands.

The mean number of years since the patients had been diagnosed with rheumatoid arthritis was 18 years (Standard Deviation of 13). However, in some cases some patients may have suffered with rheumatoid arthritis long before being diagnosed hence the disease duration may be longer than the disease duration since diagnosis. The range of years since diagnosed with rheumatoid arthritis was 41 with a minimum value of 4 and maximum value of 45. Thus, the cohort had a range of more recently diagnosed patients to patients suffering with long-term disease (see Table 7-3).

Using a visual analogue scale, subjects participating in the study were asked to describe their foot pain in the past 4 weeks on a scale from 0 (no

pain) to 10 (worst pain imaginable) by placing a vertical line on the scale (see Appendix 12). The distance of the drawn line from 0 was measured in centimetres to quantify the level of pain. The mean pain level was 5.1 centimetres (Standard Deviation of 3.1). The minimum and maximum values were 1.0 to 10.0 respectively with a range of 9.0 centimetres. This suggests that all subjects were feeling some level of pain at the time of the study with one subject suffering minimum pain to one suffering from maximum pain. However, the majority were clustered towards the middle of the scale (see Figure 7-2).

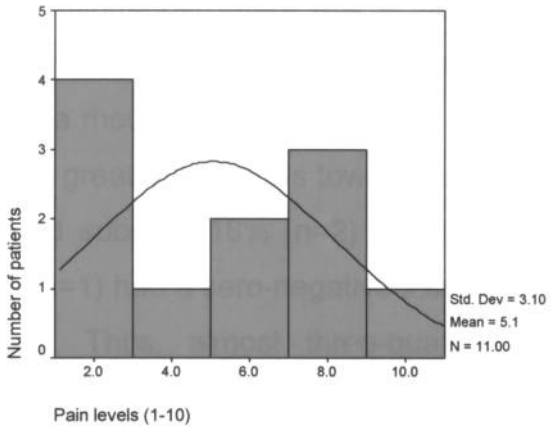


Figure 7-2: Histogram for pain levels for subjects with rheumatoid arthritis.

The erythrocyte sedimentation rate test was described in detail in Appendix 7 and gives an indication of active rheumatoid arthritis and/or temporal arteritis. The reference ranges of the Erythrocyte sedimentation rate in healthy adults are shown in Table 7-2. One male above the age of 50 years had an erythrocyte sedimentation rate of 8 millimetres per hour and was within the normal ranges. For 6 females below the age of 50 years only one had a slightly elevated erythrocyte sedimentation rate. Finally, of the females above the age of 50 years only one had a raised erythrocyte sedimentation rate. Thus overall, 18% (n=2) had slightly raised erythrocyte sedimentation rate levels and the remaining 82% (n=9) had normal levels. None had a significantly elevated erythrocyte sedimentation rate indicating active vasculitic disease.

Adults	Normal range (Millimetres per hour)
Age below 50 years	
Men	0 - 15
Women	0 - 20
Age above 50 years	
Men	0 - 20
Women	0 - 30

Table 7-2: The ranges of the erythrocyte sedimentation rate for healthy men and women (adapted from Bottiger *et al.*, 1967).

The rheumatoid factor test is used to classify and monitor patients with rheumatoid arthritis and has been described in Appendix 7. Where patients have a rheumatoid factor positive, it suggests that the disease is active and has a greater prognosis towards a more severe and destructive disease. Of the 11 subjects 18% (n=2) had not had a recent Rheumatoid Factor test, 9% (n=1) had a sero-negative test factor and 73% (n=8) had a sero-positive result. Thus, almost three-quarters of the group had been recently diagnosed with the most severe form of the disease.

7.8.3 Cohort characteristics of control subjects

Eleven healthy subjects were tested, 2 males and 9 females, with a mean age of 41 years (Standard Deviation of 11). The age range was 32 years with a minimum and maximum value of 27 and 59 respectively. The group had a mean shoe size of 6 (Standard Deviation of 1.7). The range for the shoe size was 5 with a minimum and maximum value of 4 and 9 respectively (see Table 7-3).

The mean mass of the control cohort was 69 Kilograms (Standard Deviation of 13) with a range of 39 Kilograms and a minimum value of 50 and maximum of 89. The mean height was 1.65 metres (Standard Deviation of 0.08) and the cohort had a range of 0.21 metres with a minimum and maximum value of 1.52 and 1.73 respectively. The average body mass index was 25.3 (Standard Deviation of 2.9). The range of the body mass index was 8.9 with a minimum value of 21.2 and maximum value of 30.1.

Thus, the group mean may be described as overweight ranging from normal weight to obese (see Table 7-3).

	Rheumatoid arthritis	***Controls***
Number (gender)	N=11 (1 males: 10 females)	N=11 (2 males: 9 females)
Mean age (SD*)	51 (12) years	41 (11) years
Mean mass (SD*)	75 (23) Kilograms	69 (13) Kilograms
Mean height (SD*)	1.66 (0.10) metres	1.65 (0.08) metres
Mean Body Mass Index (SD*)	27 (6.7)	25 (2.9)
Mean shoe size (SD*)	6 (2)	6 (1.7)

Table 7-3: The characteristics of the rheumatoid arthritis and control groups. *The standard deviation is shown in brackets.

7.8.4 Comparisons between rheumatoid arthritis patients and healthy controls

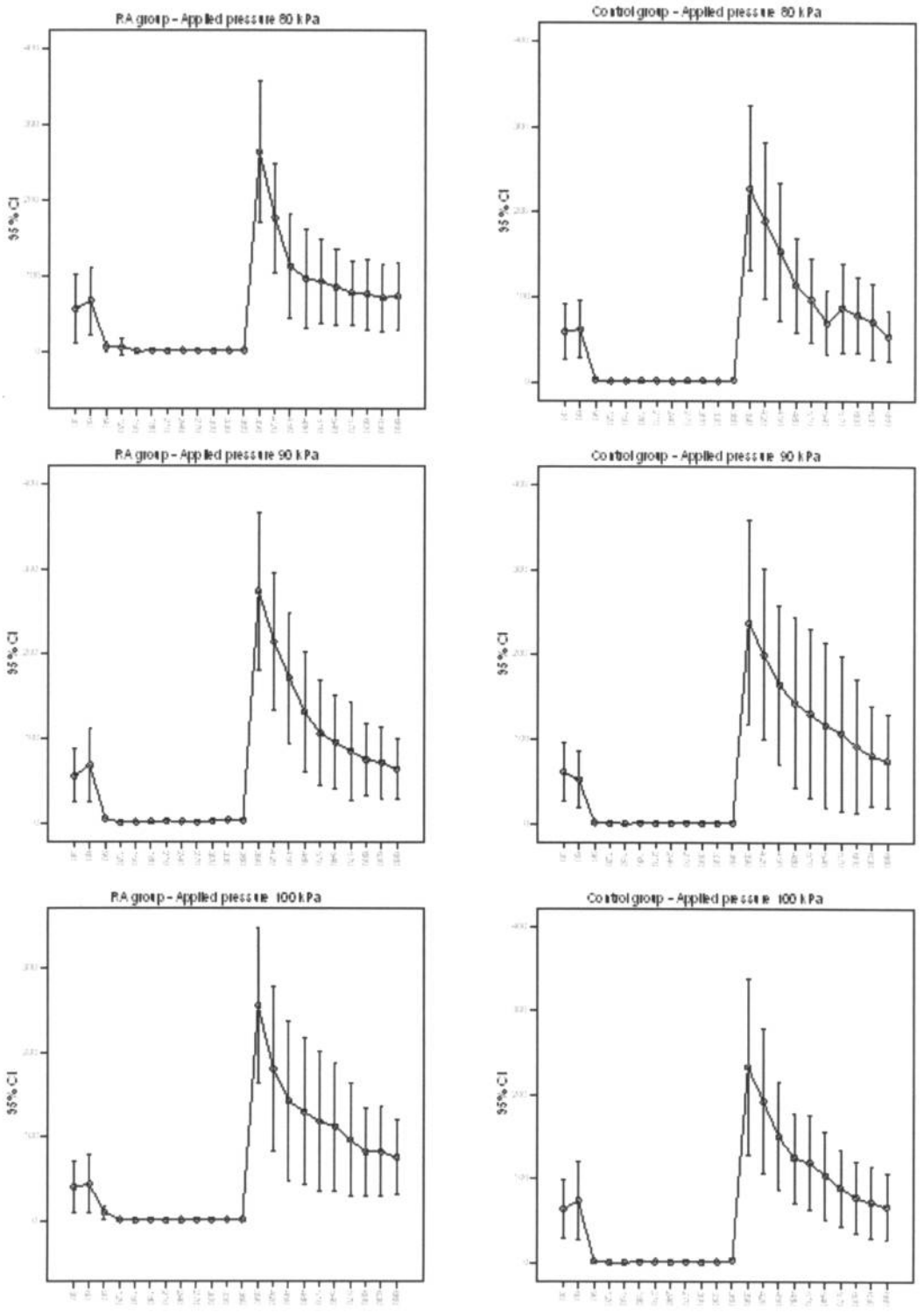


Figure 7-3: Error plot spline graphs at 95% confidence intervals for patients with rheumatoid arthritis and healthy controls. The graphs show the effects of applying 80, 90 and 100 kPa of plantar foot pressure on skin blood flow.

Figure 7-3 shows errors bars at 95% confidence interval with the means connected by a line. In all graphs the y-axis represents the laser Doppler

flux (Arbitrary Units) and the x-axis represents the time in seconds (that is, t30 equals 30 seconds, t60 equals 60 seconds, etc.). There were no significant differences between all the curves, although the rheumatoid arthritis group has a slightly higher mean hyperaemic response peak when compared to the healthy controls. Thus, further statistical analysis was carried out using a Mann-Whitney U test to investigate any differences between both groups. There were no statistically significant differences between both groups except during the application of 90 kPa of plantar foot pressure at the 90 seconds interval (see Table 7-4).

Time intervals (Seconds)	Applied pressure		
	80 kPa	90 kPa	100 kPa
30	p=0.53	p=0.58	p=0.20
	p>0.05	p>0.05	p>0.05
60	p=0.87	p=0.92	p=0.25
	p>0.05	p>0.05	p>0.05
90	p=0.10	p=0.01*	p=0.08
	p>0.05	p<0.05	p>0.05
120	p=0.41	p=0.77	p=0.53
	p>0.05	p>0.05	p>0.05
150	p=0.62	p=0.14	p=0.35
	p>0.05	p>0.05	p>0.05
180	p=0.92	p=0.08	p=0.51
	p>0.05	p>0.05	p>0.05
210	p=0.69	p=0.17	p=0.41
	p>0.05	p>0.05	p>0.05
240	p=0.19	p=0.32	p=0.79
	p>0.05	p>0.05	p>0.05
270	p=0.84	p=0.53	p=0.79
	p>0.05	p>0.05	p>0.05
300	p=0.95	p=0.21	p=0.67
	p>0.05	p>0.05	p>0.05
330	p=0.26	p=0.09	p=1.00
	p>0.05	p>0.05	p>0.05
360	p=0.95	p=0.10	p=0.37
	p>0.05	p>0.05	p>0.05
390	p=0.58	p=0.72	p=0.77
	p>0.05	p>0.05	p>0.05
420	p=0.82	p=0.82	p=0.87
	p>0.05	p>0.05	p>0.05
450	p=0.45	p=0.72	p=0.72
	p>0.05	p>0.05	p>0.05
480	p=0.49	p=0.92	p=0.87
	p>0.05	p>0.05	p>0.05
510	p=0.97	p=0.97	p=0.77
	p>0.05	p>0.05	p>0.05
540	p=0.67	p=0.62	p=0.87
	p>0.05	p>0.05	p>0.05
570	p=0.67	p=0.77	p=0.87
	p>0.05	p>0.05	p>0.05
600	p=0.82	p=0.53	p=0.82
	p>0.05	p>0.05	p>0.05
630	p=0.92	p=0.77	p=0.82
	p>0.05	p>0.05	p>0.05
660	p=0.72	p=0.77	p=0.92
	p>0.05	p>0.05	p>0.05

Table 7-4: Statistical analysis for the comparison between patients with rheumatoid arthritis and healthy controls. The Mann-Whitney U test is shown. *Statistically significant at 95% confidence interval.

7.8.5 Within subjects effects for patients with rheumatoid arthritis and healthy controls

The within subjects effects were analysed using Friedman's tests to determine if differences existed between the baseline, during the application of 80 kPa, 90 kPa or 100 kPa of plantar foot pressure and during the post-

occlusive hyperaemic response. The results for both groups suggested a significant difference between baseline, period of pressure application and hyperaemic response for all applied pressures (see Table 7-5).

Rheumatoid arthritis			*Control*		
80 kPa	***90 kPa***	***100 kPa***	***80 kPa***	***90 kPa***	***100 kPa***
p=0.000* p<0.01	p=0.000* p<0.01	p=0.000* p<0.01	p=0.000* p<0.01	p=0.000* p<0.01	p=0.000* p<0.01

Table 7-5: Statistical analysis for the within subjects effects for those with rheunmatoid arthritis and healthy controls. The Friedman's test is shown. *Statistically significant at 99% confidence interval.

7.9 Discussion

A control laser Doppler flowmeter transducer was used in both groups to ascertain that any changes occurring in the experimental transducer were due to the effects of plantar foot pressure on skin blood flow and not some other physiological response. Since in all the traces the control laser Doppler flux remained stable one may infer that any changes in skin blood flow recorded using the experimental transducer in the three-tier piston below the heel was due to the physiological micro-vascular response to plantar foot pressure. However, Freeburg *et al.* (1960) found that following arterial occlusion some local vasodilatating metabolites released by the ischaemic tissues in the leg became blood borne and had a vasodilatating effect in the forearm. An extensive literature search revealed no other articles with similar findings. This study showed that following the effects of plantar foot pressure on skin blood flow there appeared to be no blood borne mediated effect in the control probe attached to the forefoot of the same foot.

There were some differences between the rheumatoid arthritis and control cohorts especially with regards to age, with the rheumatoid arthritis having a higher mean age. With increasing age changes to the skin and its vascular supply results in a reduction in the micro-vascular supply to the skin leading to atrophy of the epidermis and thinning of the skin (Livingston, 1992; Ryan, 1966). Hence, the problems encountered with matching the cohorts for age

favours the rheumatoid arthritis cohort to having a more dysfunctional circulatory response to plantar foot pressure when compared to the control group. From the inclusion criteria none of the cohorts had evidence of micro-vascular disease or were taking medication that would affect it. With regards to the rheumatoid arthritis group none had a history of or active vasculitis or peripheral neuropathy at the time of the study.

Previous authors have described the laser Doppler fluxmeter curve before, during and after arterial acclusion (see Chapter 5). The within subjects effects was studied for the three parts of the curve which were statistically analysed for each group at all three pressures. A statistically significant difference was found between the baseline, during the application of each pressure and during the resulting post-occlusive hyperaemic response. Physiologically these three phases can also be differentiated. Following the application of plantar foot pressure a decrease in skin blood flow from baseline values occurs. This causes a decrease or total arrest in skin blood flow depending on applied pressure. Whether partial or total arrest is caused it is dependant on the intravascular pressure of the vessels located in the area were plantar foot pressure is applied. Beer (1971) found that the higher the intravascular pressure the higher the extravascular pressure needed to collapse the blood vessel. Following the release of plantar foot pressure a hyperaemic response occurs. The duration and magnitude of this response is proportional to the occlusive insult. Some authors have attributed the post-occlusive hyperaemic response to the metabolic debt (that is, the reduction in supply of oxygen to local tissues) and the build-up of local metabolites that occurred during the circulatory reduction/arrest (see sections 3.10 Pathophysiology of the skin and 3.11 Mechanical pressure, skin blood flow and tissue oxygen) (Colin *et al.,* 1996; Kabagambe *et al.,* 1994; Livingston, 1992; Hagisawa *at al.,* 1991; Ek *et al.,* 1984; Bauman *et al.,* 1963; Carrier *et al.,* 1964; Ashton, 1962[a]; Ross *et al.,* 1962; Crawford *et al.,* 1959).

There were no significant differences between baseline laser Doppler flux values for the control and rheumatoid arthritis groups. The response of skin

blood flow to the application of plantar foot pressure indicated no significant differences except in the 90 seconds interval when 90 kPa of plantar foot pressure was being applied. This statistically significant difference may have occurred due to motion artifarct as a result of the soft tissues being compressed rather than a physiological difference between both groups.

The glaborous skin in healthy individuals tends to be thicker than in other parts of the body. However, in patients with rheumatoid arthritis with disease progression and subsequent treatment (for example, steroids) the skin atrophies and becomes thinner, in some cases almost papery (Braid, 1996; Geppert *et al.,* 1992). In addition degeneration of the fat pad and changes in the constitution of the fat reduces the ability of the fat pad to absorb and dissipate energy during ambulation (Resnick *et al.,* 1999; Jacobsson *et al.,* 1990). In the forefoot this is compounded by forward shift of the fat pad exposing bony prominences. Deformity of the foot, a result of the joint changes, further affects function and the result is a foot with high plantar foot pressure. For applied pressure to cause a disruption of the normal physiological function of living cells or death the pressures would have to be extreme (Cattell, 1936). These extreme pressures are beyond that found in the 'normal' or rheumatoid foot hence a different mechanism that leads to cellular death, soft tissue breakdown and ulceration must exist. Thus, it is more likely that plantar foot ulcers develop following low pressure over a prolonged period of time or high pressure over a short period of time by depriving the tissues of its nutritive blood supply and causing cellular starvation leading to death and soft tissue breakdown (Colin *et al.,* 1996; Kabagambe *et al.,* 1994; Livingston, 1992; Ek *et al.,* 1984; Bauman *et al.,* 1963). However despite this, plantar foot ulcers are less common than in patients with diabetes mellitus. Capillary collapse or closure of the vessel occurs when the tissue pressure exceeds the intra-vascular pressure (Ashton, 1975; Ashton, 1963; Burton, 1951). If at the skin's microcirculation level the intra-vascular pressure is raised this could explain the statistical difference between the control and rheumatoid arthritis group during pressure application with the rheumatoid arthritis group being slightly more resistant to the application of pressure. This may serve as a protective

function maintaining the nutritive blood supply to the skin a little longer and hence cope with the insults of the higher pressure and prevent tissue starvation and breakdown in those patients. Studies have found that in patients with peripheral arterial occlusive disease the nutritive capillaries close at a lower external pressure hence are more prone to mechanically mediated soft tissue breakdown following cellular starvation and death at these lower pressures (Fisher *et al.,* 1995; Castronuovo *et al.,* 1987). However, a limitation of the study was that no patients with active vasculitis were tested.

There was no significant difference between the control and rheumatoid arthritis group response to vascular insult, that is, the hyperaemic response following the release of plantar foot pressure. The post-occlusive hyperaemic response is due to the metabolic dept caused by the reduction/arrest of skin blood flow (Kabagambe *et al.,* 1994; Hagisawa *et al.,* 1991). Using acetylcholine and the laser Doppler flowmeter in 8 rheumatoid arthritis subjects, 5 with a high C-reactive protein and 3 with a low C-reactive protein, Ferrell *et al.* (2001) found evidence of impaired endothelium-dependant dilatation in the high C-reactive protein group. Thus, endothelium-dependant dilatation is markedly impaired in patients with acute flare-up. However, in the present study no patients showed signs of acute flare-up or active vasculitis and this may explain the no significant differences found between both groups.

7.10 Conclusion

The aim was accomplished by demonstrating that the effects of plantar foot pressure on skin blood flow between patients with rheumatoid arthritis (with no evidence of micro-vascular disease) and healthy controls was the same despite rheumatic changes to the foot that results in higher plantar foot pressure during ambulation. This suggests that the microcirculation is able to react quite normally to the application of plantar foot pressure preventing soft tissue injury. However, in some cases where the plantar foot pressure is extreme or vasculitis is present soft tissue break down may occur. In addition, the fact that there was a significant difference between both

groups during the application of pressure with the rheumatoid group being more resistant to circulatory arrest suggests that this may allow such patients to resist the pathogenic effects of higher plantar foot pressure.

7.11 Limitations and recommendations

A limitation of this study relates to the matching of the two groups for age.

The study has raised some important issues with regards to tissue viability and the rheumatoid foot. However a further study is recommended with four groups, healthy controls; patients with rheumatoid arthritis and no evidence of vascular disease; patients with rheumatoid arthritis and evidence of vasculitis; and patients with rheumatoid arthritis and a plantar foot ulcer. Such study would contribute new knowledge with regards to whether the vasculitis and/or ulcer group has significantly different responses to the application of plantar foot pressure on skin blood flow. If so, then the reactions of skin blood flow to the application of plantar foot pressure may be an important factor in the development of soft tissue breakdown.

CHAPTER 8

GENERAL CONCLUSIONS

7. CHAPTER 8: Overall conclusions

8.1 Introduction

In this study a device was developed and validated to measure the effects of plantar foot pressure on skin blood flow. In addition, this study has contributed to current knowledge by investigating the effects of plantar foot pressure on skin blood flow in healthy individuals in two positions (that is, supine and semi-weight bearing) and in patients with rheumatoid arthritis. The device developed in Chapter 4 was only visually synchronised, however further modifications were made in Chapter 6 and by using a synchronisation box and changing the strain gauge pressure transducer to the Novel pressure transducer the system was electronically synchronised. The developed device was designed to be mobile and hence was tested before and after dismantling, packing it, transporting by road and re-assembling. The device has shown to be robust, accurate, repeatable and reproducible for the intended experiments and suitable to measure the effects of plantar foot pressure on skin blood flow.

It is anticipated that the developed device may be used to improve current knowledge in the field of tissue viability by investigating how plantar foot pressure affects/disturbs skin blood flow. Evidence with regards to how plantar foot pressure affects skin blood flow is scant and limited since only a few experimental devices have been capable of measuring plantar foot pressure and skin blood flow simultaneous and hence investigations on how the microcirculation reacts to skin blood flow has been limited. The new device was tested on healthy subjects and patients with rheumatoid arthritis and could be used in other conditions like diabetes mellitus.

The study's aim and objectives set out in the "General Introduction" (Chapter 1; Sections 1.2 and 1.3) have been met and a number of articles published (see Appendix 14). The following sections are a summary of the overall results of the study based on the aim and objectives.

8.2 Aims

A device was developed and used to investigate, contribute and strengthen current knowledge of the effects of plantar foot pressure on skin blood flow in patients with rheumatoid arthritis.

8.3 Objectives

A device was developed to measure the effects of plantar foot pressure on skin blood flow. The device was made up of two commercially available systems, the laser Doppler fluxmeter DRT4 and the Novel pressure system. An integrated transducer piston mechanism collected data from the same site simultaneously for the variables of plantar foot pressure and skin blood flow. Both systems were electronically synchronised used a developed midway synchronisation box. A system to place analogue electronic markers on the laser Doppler fluxmeter tracers was also developed allowing the researcher to mark specific events whilst being located a distance from the computer.

The developed system was validated for use as a mobile system since it was intended to use the device in various sites. The system was tested for accuracy, repeatability and reproducibility. The range, limit of quantification and limit of detection of the system were also established.

The developed device was used to investigate the effects of plantar foot pressure on skin blood flow in various positions. The study concluded that due to the effects of the postural vascular response there was a difference between the response of skin blood flow to plantar foot pressure depending on the measuring position (that is, supine or semi-weight bearing). Thus any studies done with the subjects in a position other than upright does not reflect how the microcirculation of the skin reacts to the application of plantar foot pressure during walking. Hence, only in studies where subjects have

been tested in an upright position can inferences be may as to how skin blood flow reacts to plantar foot pressure during ambulation.

The developed device was also used to investigate the effects of plantar foot pressure on skin blood flow in patients with rheumatoid arthritis compared to a healthy control group. The subject concluded that there was no difference between both groups other than when pressure was applied. It is important to note that the rheumatoid arthritis participants had no evidence of active vasculitis (micro-vascular disease) at the time of the study. During the application of plantar foot pressure the microcirculation of the patients with rheumatoid arthritis appeared more resistant to circulatory arrest suggesting a higher intra-vascular blood pressure when compared to the healthy control group. This raised microcirculatory blood pressure may make skin blood flow more resistant to pressure by maintaining circulation to the soft tissues open for longer.

8.4 Suggestions for future research

8.4.1 Further development of the developed device

The device should be further developed with the spindle mechanism replaced with an electronically controlled pneumatic piston allowing for automatic application and removal of pressure. In addition, the pneumatic piston could be set to apply intermittent pressure simulating ambulation.

By improving the stability of the platform system static weight bearing measurements of skin blood flow could be taken.

The dynamic effects of plantar foot pressure on skin blood flow could be investigated by integrating the laser Doppler fluxmeter fibre optic transducer into the Novel Pedar insoles. Both systems can be electronically synchronised with the developed midway synchronisation box.

8.4.2 Further studies in rheumatoid arthritis

The study carried out could be improved by comparing four groups instead of the two groups studied. The proposed groups could include a healthy control group; a group with rheumatoid arthritis and no signs of vascular disease; a group with rheumatoid arthritis and active vasculitis; and a group with rheumatoid arthritis and a history of current or past plantar foot ulceration. Such study would contribute to the current study on how plantar foot pressure affects skin blood flow in rheumatoid arthritis.

8.4.3 Further studies in other conditions

The developed system could be used to study the effects of plantar foot pressure on skin blood flow in other conditions where there is a risk of developing plantar foot ulcers like diabetes mellitus.

APPENDIX 1

8. APPENDIX 1: General exclusion criteria and experimental measures taken to reduce variables that may affect skin blood flow.

9.1 General subject exclusion criteria

Subjects taking vasoactive drugs, either oral or topical or both since this may affect blood vessel function.

Calloused areas over sites were skin blood flow and plantar foot pressure is recorded since the thicker dry skin would affect the penetration depth of the laser and also affect the deforming properties of the skin.

Subjects who have consumed:

- Alcohol within 24 hours prior to data recording.
- Caffeine within 6 hours prior to data recording.
- Food within 2 hours prior to data recording.

Subjects who have carried out exercise activity 1 hour prior to the study.

Subjects who smoke cigarettes / cigars / pipe.

9.2 General experimental measures taken to reduce variables that may affect skin blood flow.

Subjects will acclimatise in a draft free room with dimmed lights for 20 minutes to reach a stable baseline skin blood flow condition.

All mobile phones will be switched off during data collection.

Researcher will ensure that there is no background environmental vibration noise during data collection.

Room temperature will be maintained at 20 to 25 °C.

Room humidity levels will be maintained at 40 ± 5%.

Subjects will be advised to:

1.1. Remove heavy clothing since if subject is too warm skin blood flow responses may be affected.

1.2. Breathe normally during data collection.

1.3. Not to take deep inspirations during data collection.

APPENDIX 2

9. APPENDIX 2: Preliminary investigations

10.1 Preliminary investigation 1: The effects of in-shoe plantar foot pressure on skin blood flow using the laser Doppler fluxmeter.

10.1.1 Aim

To investigate the suitability of the laser Doppler fluxmeter to measure the effects of in-shoe external pressure on skin blood flow with the subject in a supine and standing position.

10.1.2 Method

The P10k probe was used for the study connected to the laser Doppler fluxmeter DRT4 system using the DP10M "master" interface. Two insoles were cut out of leatherboard 1.5 mm thick. A hole 3 mm in diameter was drilled in the centre of the heel of the insoles. The probe tip disk was fitted into the hole and stuck in place, flash with the upper edge. The P10k optic fibre was then connected to the DRT4 laser Doppler fluxmeter using the DP10M "master" interface. The leatherboard insoles with the P10k probe were then placed in the subject's shoes.

Room temperature was maintained at 22.4 ±0.8°C. Three subjects were recruited. Advice described in Appendix 1 was followed.

Subjects were asked to insert their bare foot in the shoe. Following the acclimatisation period in the supine position a baseline recording was taken. The subject was then asked to sit upright with their legs in dependency but not touching the floor and a second recording taken. The subject then stood for five minutes and the episode recorded. Finally, the subject was asked to sit and the hyperaemic sequence recorded for ten minutes.

10.1.3 Results and Discussion

	Baseline (% difference between baseline and supine values)[8]	***Standing (% reduction from semi-weight bearing baseline values)***	***Maximum % increase from semi-weight bearing baseline values***
Subject 1	26	-16	48
Subject 2	34	-24	64
Subject 3	38	-25	62

Table 9-1: The effects of in-shoe plantar foot pressure on skin blood flow (flux) are shown. The first column shows the percentage difference between the baseline recordings in the supine and semi-weight bearing positions. The second column shows the percentage reduction in skin blood flow from the semi-weight bearing baseline measurement. The third column shows the maximum percentage increase in skin blood flow during the hyperaemic response from the baseline semi-weight bearing value. All and are in perfusion arbitrary units.

Differences were detected between baseline recordings with the subject in a non-weight bearing position in the supine and sitting upright positions. This

[8] This refers to the venoarteriolar response (VAR) and is calculated by the following formula: SBF means skin blood flow.

$$VAR = \frac{SupineSBF - SemiWeightBearingSBF}{SupineSBF} \times 100$$

suggests that the laser Doppler fluxmeter system is sufficiently sensitive enough for detecting differences in skin blood flow caused by the venoarteriolar response. Differences between the two positions in baseline recordings may suggest that the physiological response of the microcirculation of the skin to plantar foot pressure may differ, however a study would need be carried out to confirm this.

On weight bearing a marked reduction in skin blood flow was observed, which was followed by a marked increase in skin blood flow upon removing the plantar foot pressure and sitting in an upright position. This marked increase in skin blood flow took several minutes to settle back to its baseline values. Thus the laser Doppler fluxmeter is sufficiently sensitive to measure the effects of plantar foot pressure on skin blood flow.

10.2 Preliminary investigation 2: A system to quantify and measure the effects of plantar foot pressure on skin blood flow.

10.2.1 Aim

To investigate and develop a system, measure and quantify, the effects of plantar foot pressure on skin blood flow during standing.

10.2.2 Method

The laser Doppler fluxmeter system with the leatherboard insole was used together with the Pedar standard plantar foot pressure measuring system (Novell, UK).

The Pedar insole was placed in the shoe beneath the leatherboard insole with the laser Doppler fluxmeter sensor. A double-sided adhesive ring was place around the skin blood flow sensor and the subject was asked to place their bare foot in the shoe. All cables were fixed to the legs using Velcro® straps to prevent any movement artefact arising during the recording sessions. No electronic synchronisation between the Pedar standard plantar foot pressure and laser Doppler fluxmeter systems was possible.

Two subjects were recruited for this study.

The subjects acclimatised in a sitting position with the legs in dependency, without touching the floor, and the standard advice described in the Appendix 1 given. Following the acclimatisation period the subject was asked to stand and the Pedar standard pressure sensors reset to zero as recommended by the manufacturer. Recording of plantar foot pressure and skin blood flow then commenced. First a baseline recording by lifting the measuring foot was measured, followed by a five minute standing recording, proceeded by lifting the measuring foot and measuring skin blood flow back to baseline from the hyperaemic response.

10.2.3 Results and Discussion

	Protocol One	
	Standing (% reduction from semi-weight bearing baseline values)	*Maximum % increase from semi-weight bearing baseline values*
Subject 1	-12	42
Subject 2	-21	51

Table 9-2: Effects of in-shoe plantar foot pressure on skin blood flow (flux). The first column shows the percentage reduction in skin blood flow from the semi-weight bearing baseline measurement. The second column shows the maximum percentage increase in skin blood flow during the hyperaemic response from the baseline semi-weight bearing value. All the values related to the measurement of laser Doppler fluxmeter flux and are in perfusion arbitrary units.

The results were very positive with reductions and increases in skin blood flow observed during periods of weight bearing and non-weight bearing. However, the clearly demarcated areas of higher pressure relative to the rest of the heel along the area were resin glue had been applied was observed. Thus the insole system would have to be redesigned if this system is to be used. A limitation of this system is that the plantar foot pressure measured are representative of the insole to Pedar insole rather than of the foot to pressure insole.

10.3 Preliminary investigation 3: Another system to quantify and measure the effects of plantar foot pressure on skin blood flow.

10.3.1 Aim

To investigate and develop a modified system, to measure and quantify, the effects of plantar foot pressure on skin blood flow.

10.3.2 Method

A study was carried out to investigate if the above method could be adapted. The same two measurement protocols used above were adopted, however, skin blood flow was measured first. The leatherboard insole was then removed and the Pedar standard plantar foot pressure insole inserted. All cables and electronic boxes were attached as described earlier and the pressure recorded for the two protocols.

10.3.3 Results and Discussion

	Standing (% reduction from semi-weight bearing baseline values)	*Maximum % increase from semi-weight bearing baseline values*
Subject 1	-32	57
Subject 2	-28	48
Subject 3	-36	61

Table 9-3: Effects of in-shoe plantar foot pressure on skin blood flow. The first column shows the percentage reduction in skin blood flow from the semi-weight bearing baseline measurement. The second column shows the maximum percentage increase in skin blood flow during the hyperaemic response from the baseline semi-weight bearing value.

During the analysis identifying in the pressure map the location of the laser Doppler fluxmeter sensor was difficult. Also since the systems were not synchronised and were part of two separate recordings (especially during the walking protocol) the study concluded that such option was not viable. Following this study other options were explored. The best option involved drilling a hole thorough the capacitive Pedar pressure sensor and feeding the laser Doppler fluxmeter fibre optic sensor through the hole and adhering it flush with the skin surface of the pressure sensor. However such option proved too expensive, as it would have involved damaging a costly insole for a pilot study and it was decided not to carry out such a pilot. Another option consisted of embedding a laser Doppler fluxmeter skin probe and a pressure sensor in a piston system that would enable quantifiable plantar foot pressure to be applied to the skin and its effect on the microcirculation measured. The second option was recommended for further investigation.

10.4 Preliminary investigation 4: A piston mechanism to investigate the effects of quantifiable plantar foot pressure on skin blood flow.

10.4.1 Aim

The aim of this study was to investigate the suitability of a piston mechanism to investigate the effects of quantifiable plantar foot pressure on skin blood flow in the supine position.

10.4.2 Method

A strain gauge (type LM-50KA, Kyowa Electronic Instruments Ltd., Japan) was chosen to measure external pressure. The strain gauge was adhered to a wooden shaft

with a hole that was drilled from the skin surface of the shaft to accommodate the laser Doppler fluxmeter skin probe (type DP1T-V2, Moor Instruments Ltd, UK). The skin probe measures skin blood flow and temperature. A double-sided adhesive ring was stuck on the skin side of the shaft around the laser Doppler fluxmeter sensor to ensure skin contact was maintained throughout the pilot.

Two subjects were recruited for the study.

Acclimatisation and advice was given as in the previous studies.

Following acclimatisation in the supine position the skin end of the shaft was held against the skin and a baseline recording taken. External pressure was applied and quantified whilst simultaneously recording skin blood flow for three minutes. The external pressure was removed, confirmed by ensuring the strain gauge was reading zero, and the hyperaemic response recorded until the signal restored to baseline.

10.4.3 Results and Discussion

	% reduction from supine baseline values	*Maximum % increase from supine baseline values*
Subject 1	-20	39
Subject 2	-14	37

Table 9-4: Effects of applied plantar foot pressure on skin blood flow in the supine position. The first column shows the percentage reduction in skin blood flow from the supine baseline measurement. The second column shows the maximum percentage increase in skin blood flow during the hyperaemic response from the baseline supine value.

The results showed that at baseline with measurable zero plantar foot pressure skin blood flow and temperature can be recorded. Upon the application of external pressure, that could be quantified, the effects of a given pressure upon skin blood flow and temperature could also simultaneously be recorded. Provided the system could be incorporated into a shoe and able to measure in the supine and semi-weight bearing upright positions, the effects of quantifiable plantar foot pressure on skin blood flow can be measured.

APPENDIX 3

10. APPENDIX 3: Development of a computerised conversion calculator for mass / voltage to force / pressure

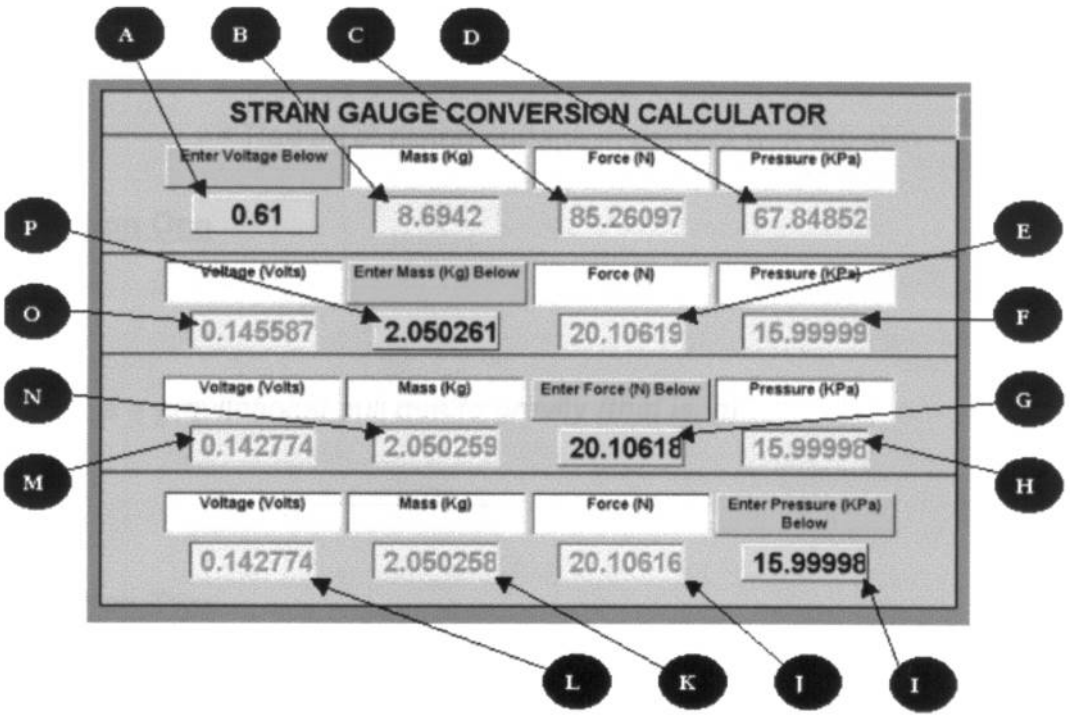


Figure 10-1: The strain gauge conversion calculator is shown above. By entering a figure in a bright orange box the correct conversion appears in the light orange boxes of the same line of the respective bright orange box. The boxes have been labeled with a letter A to P to allow easy identification of the background equation.

A Microsoft excel module was written with background equations. To facilitate what equation exists in the background of each box, each box has been labelled with a letter from A to P.

Where, F = Force

m = Mass

a = Acceleration

g = Gravitation pull due to earth

P = Pressure

A = Surface area of piston in skin contact

y = Following regression, the point where the line crosses the chart's y axis

c = Regression Constant

v = Voltage output from strain gauge following calibration against know masses

r = Radius of piston surface in contact with the skin

Equation One

$$F = ma$$

If *a = gravitational pull due to gravity (that is, g)*

Then, $F = mg \xrightarrow{EquationOne}$

Equation Two

$$P = \frac{F}{A} \xrightarrow{EquationTwo}$$

Equation Three

Substitute Equation One in Equation Two,

$$P = \frac{mg}{A} \xrightarrow{EquationThree}$$

Equation Four

From regression formula following correlation between applied pressures used to calibrate strain gauge and voltage output of strain gauge.

$$v = cP + y \xrightarrow{EquationFour}$$

Equation Five

From Equation Four,

$$v = cP + y$$

$$\therefore v - y = cP$$

So,

$$P = \frac{v - y}{c} \xrightarrow{EquationFive}$$

Equation Six - Three-tier Piston Surface Area

Using equation,

Then, $PistonSurfaceArea = \pi r^2$

If, $r = 0.02$ *metres*

Then, $PistonSurfaceArea = \pi(0.02)^2 = 0.0004\pi \xrightarrow{EquationSix}$

Background equations in Strain Gauge Calculator as shown in Figure 10-1

Box (A) – this box is an input box for voltage output from strain gauge and has no background equation

Box (B) – the equation in Box (B) was calculated as follows:

Using equation three, $P = \frac{mg}{A}$

Substitute equation five in equation three, $\frac{v - y}{c} = \frac{mg}{A}$

Therefore, $m = \dfrac{A(v-y)}{gc}$

Box (C) – the equation in Box (C) was calculated as follows:

Using Equation One, $F = mg$

To calculate force using voltage output from strain gauge, substituting Equation 4 in Equation One,

$F = g(y + cv)$

Box (D) – this box uses Equation Five to calculate the applied Pressure using the voltage value entered in box (A)

Box (E) – this box uses Equation One to calculate the applied force using the mass value entered in box (P)

Box (F) – this box uses Equation Three to calculate the applied pressure using the mass value entered in box (P)

Box (G) – this box is an input box for applied force and has no background equation

Box (H) – this box uses Equation Two to calculate the applied pressure using the applied force value entered in box (G)

Box (I) – this box is an input box for applied pressure and has no background equation

Box (J) - the equation in Box (J) was calculated as follows:

Using Equation Two, $P = \dfrac{F}{A}$

$\therefore F = PA$

Box (K) – the equation for Box (K) was calculated as follows:

Using Equation Three, $P = \frac{mg}{A}$

$mg = PA$

$\therefore m = \frac{PA}{g}$

Box (L) – the equation for Box (L) was calculated as follows:

Using Equation Five, $P = \frac{v - y}{c}$

$cP = v - y$

$\therefore v = cP + y$

Box (M) – the equation for Box (M) was calculated as follows:

To calculate the voltage output from the strain gauge using the applied force value entered in Box (G),

Using Equation Two, $P = \frac{F}{A}$

Substituting Equation Five in Equation Two,

$\frac{F}{A} = \frac{v - y}{c}$

$V = \frac{cF}{A} + y$

Box (N) – the equation for Box (N) was calculated as follows:

To calculate mass using force value entered in Box (G),

Using Equation One, $F = mg$

$$\therefore m = \frac{F}{g}$$

Box (O) – the equation for Box (O) was calculated as follows:

To calculate voltage output from strain gauge using mass value entered in Box (P),

Using Equation Four and Three, $v = \frac{c(mg)}{A} + y$

Box (P) – this box is an input box for mass and has no background equation

APPENDIX 4

11. APPENDIX 4: Within analysis for strain gauge

12.1 The within measurements analysis of repeatability for the strain gauge.

12.1.1 Summary of repeatability results for loading measurements taken before dismantling, transportation and reassembly of equipment.

Applied load	Median	Minimum	Maximum	Percentiles		IQR	Percentile		90% range
				25th	75th		5th	95th	
0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	0	1	1	0
1	1	1	1	1	1	0	1	1	0
1	1	1	2	1	2	1	1	2	1
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
3	3	3	3	3	3	0	3	3	0
2	2	2	4	2	3	1	2	4	2
4	4	4	5	4	4	0	4	5	1
5	5	5	5	5	5	0	5	5	0
9	9	9	10	9	9	0	9	10	1
13	13	13	13	13	13	0	13	13	0
17	17	17	18	17	17	0	17	18	1
21	21	21	22	21	21	0	21	22	1
25	25	25	26	25	25	0	25	26	1
30	29	29	30	29	29	0	29	30	1
33	33	33	33	33	33	0	33	33	0
37	37	37	38	37	37	0	37	38	1
44	45	44	45	44	45	0	44	45	1
49	49	49	50	49	50	1	49	50	1
53	53	53	54	53	53	0	53	54	1
56	56	56	57	56	57	1	56	57	1
59	60	59	61	59	60	0	59	61	2
65	65	64	65	64	65	0	64	65	1
69	70	69	71	69	70	1	69	71	2
73	73	72	74	72	73	1	72	74	2
77	77	77	79	77	77	0	77	78	1
79	79	79	81	79	79	0	79	80	1
83	84	83	85	83	84	1	83	85	2
89	89	87	90	88	89	0	87	90	2
92	93	92	94	92	93	0	92	94	2
96	96	95	97	95	96	0	95	97	2
100	99	99	100	99	100	1	99	100	1

Table 11-1: The analysis of within measurements for the repeatability experiment for loading before dismantling, transportation and reassembly of the equipment. The analysis relates to 7 repeated measurements. All data is in kilo-Pascals.

12.1.2 Summary of repeatability results for unloading measurements taken before dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentile 5th	Percentile 95th	90% range
100	99	99	100	99	100	1	99	100	1
96	96	95	97	95	96	0	95	97	2
93	93	92	94	92	93	0	92	94	2
87	89	87	90	87	89	1	87	90	3
84	84	83	85	83	84	1	83	85	2
79	79	79	80	79	79	0	79	80	1
76	77	76	79	76	77	0	76	78	2
73	73	72	74	72	73	1	72	74	2
70	69	69	71	69	70	1	69	71	2
64	65	64	65	64	65	1	64	65	1
59	59	59	61	59	60	1	59	61	2
57	56	56	57	56	57	1	56	57	1
53	53	53	54	53	53	0	53	54	1
49	49	49	50	49	49	0	49	50	1
45	45	44	45	44	45	1	44	45	1
37	37	37	38	37	37	0	37	38	1
33	33	33	33	33	33	0	33	33	0
29	29	29	30	29	29	0	29	30	1
26	25	25	26	25	25	0	25	26	1
21	21	21	22	21	22	1	21	22	1
17	17	17	18	17	17	0	17	18	1
13	13	12	13	13	13	0	12	13	1
9	10	9	10	9	10	1	9	10	1
6	5	5	6	5	5	0	5	6	1
5	4	4	5	4	4	0	4	5	1
3	3	3	4	3	4	1	3	4	1
3	3	2	3	2	3	0	2	3	1
2	2	2	3	2	2	0	2	3	1
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
1	1	1	2	1	1	0	1	2	1
2	1	1	2	1	1	0	1	2	1
1	1	1	1	1	1	0	1	1	0
0	0	0	0	0	0	0	0	0	0

Table 11-2: The analysis of within measurements for the repeatability experiment for unloading before dismantling, transportation and reassembly of the equipment. The analysis relates to 7 repeated measurements. All data is in kilo-Pascals.

12.1.3 Summary of repeatability results for loading measurements taken after dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles		IQR	Percentile		90% range
				25th	75th		5th	95th	
0	0	0	0	0	0	0	0	0	0
1	1	1	2	1	2	1	1	2	1
1	1	1	2	1	1	0	1	2	1
2	2	1	2	1	2	0	1	2	1
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	3	2	2	0	2	3	1
3	2	2	3	2	2	0	2	3	1
3	3	3	3	3	3	0	3	3	0
3	3	3	4	3	4	1	3	4	1
4	4	4	5	4	4	0	4	5	1
5	5	5	6	5	5	0	5	6	1
9	9	9	10	9	9	0	9	10	1
13	13	12	13	12	13	0	12	13	1
18	17	17	18	17	17	0	17	18	1
21	22	21	23	21	22	1	21	23	2
25	25	25	27	25	26	1	25	27	2
30	30	29	30	29	30	0	29	30	1
33	33	32	33	32	33	0	32	33	1
37	37	36	38	37	38	1	36	38	2
45	45	44	45	44	45	0	44	45	1
49	49	49	50	49	49	0	49	50	1
54	53	53	54	53	53	0	53	54	1
56	56	56	57	56	57	1	56	57	1
60	60	59	61	60	60	0	59	61	2
64	64	64	65	64	65	1	64	65	1
69	69	69	71	69	70	1	69	71	2
73	73	72	74	72	73	1	72	74	2
76	77	76	79	76	77	1	76	78	2
80	80	79	81	79	80	1	79	81	2
83	84	83	85	84	84	0	83	85	1
89	89	88	90	89	89	0	88	90	1
93	93	92	94	92	93	0	92	94	2
96	96	95	97	96	96	0	95	97	2
100	100	99	100	99	100	1	99	100	1

Table 11-3: The analysis of within measurements for the repeatability experiment for loading after dismantling, transportation and reassembly of the equipment. The analysis relates to 7 repeated measurements. All data is in kilo-Pascals.

12.1.4 Summary of repeatability results for unloading measurements after before dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles		IQR	Percentile		90% range
				25th	75th		5th	95th	
100	99	99	100	99	100	1	99	100	1
97	96	95	97	96	96	0	95	97	2
93	93	92	94	93	93	0	92	94	1
88	89	87	90	88	89	1	87	90	3
84	84	83	85	83	84	0	83	85	2
78	79	78	80	79	79	0	78	80	2
76	76	76	77	76	76	0	76	77	1
73	73	72	73	72	73	0	72	73	1
70	70	69	71	69	70	0	69	71	2
65	65	64	65	64	65	1	64	65	1
60	59	59	60	59	60	1	59	60	1
57	57	56	57	56	57	0	56	57	1
53	53	53	53	53	53	0	53	53	0
49	49	49	50	49	49	0	49	50	1
45	45	44	45	44	45	1	44	45	1
37	37	37	38	37	37	0	37	38	1
33	33	32	33	32	33	0	32	33	1
29	29	28	30	29	30	1	28	30	2
26	26	25	26	25	26	1	25	26	1
22	22	21	22	21	22	0	21	22	1
17	17	16	18	17	17	0	16	18	1
12	13	12	13	12	13	1	12	13	1
9	9	9	10	9	9	0	9	10	1
6	5	5	6	5	6	1	5	6	1
4	4	4	5	4	4	0	4	5	1
4	4	3	4	3	4	1	3	4	1
3	3	3	3	3	3	0	3	3	0
2	2	2	3	2	2	0	2	3	1
2	2	2	3	2	2	0	2	3	1
2	2	2	3	2	2	0	2	3	1
2	2	2	3	2	2	0	2	3	1
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	1	2	2	2	0	1	2	1
1	1	1	2	1	1	0	1	2	1
1	1	1	1	1	1	0	1	1	0
1	1	1	1	1	1	0	1	1	0
0	0	0	0	0	0	0	0	0	0

Table 11-4: The analysis of within measurements for the repeatability experiment for unloading after dismantling, transportation and reassembly of the equipment. The analysis relates to 7 repeated measurements. All data is in kilo-Pascals.

12.2 The within measurements analysis of equipment warm-up reproducibility for the strain gauge.

12.2.1 Summary of equipment warm-up reproducibility results for loading measurements taken before dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles		IQR	Percentile		90% range
				25th	75th		5th	95th	
0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	0	1	1	0
1	1	1	1	1	1	0	1	1	0
2	1	1	2	1	1	0	1	2	1
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
3	2	2	3	2	2	0	2	3	1
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
3	3	3	3	3	3	0	3	3	0
4	4	4	4	4	4	0	4	4	0
4	4	4	4	4	4	0	4	4	0
5	5	5	5	5	5	0	5	5	0
10	9	9	10	9	9	0	9	10	1
13	13	12	13	13	13	0	12	13	1
17	17	17	17	17	17	0	17	17	0
21	21	21	21	21	21	0	21	21	0
25	25	25	25	25	25	0	25	25	0
29	29	29	30	29	29	0	29	30	1
33	33	33	33	33	33	0	33	33	0
38	37	37	38	37	37	0	37	38	1
45	45	44	45	45	45	0	44	45	1
50	49	49	50	49	49	0	49	50	1
53	53	53	53	53	53	0	53	53	0
57	57	56	57	57	57	0	56	57	1
60	60	60	60	60	60	0	60	60	0
65	65	64	65	65	65	0	64	65	1
70	70	69	70	70	70	0	69	70	1
73	72	72	73	72	73	1	72	73	1
77	77	77	77	77	77	0	77	77	0
80	79	79	80	79	79	0	79	80	1
85	84	83	85	84	85	1	83	85	2
89	89	89	89	89	89	0	89	89	0
92	92	92	93	92	93	1	92	93	1
96	96	95	96	96	96	0	95	96	1
99	99	99	100	99	99	0	99	100	1

Table 11-5: The analysis of within measurements for the equipment warm-up reproducibility experiment for loading before dismantling, transportation and reassembly of the equipment. The analysis relates to 4 repeated measurements at 1-hour interval. All data is in kilo-Pascals.

12.2.2 Summary of equipment warm-up reproducibility results for unloading measurements taken before dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles		IQR	Percentile		90% range
				25th	75th		5th	95th	
99	99	99	99	99	99	0	99	99	0
95	95	95	96	95	96	1	95	96	1
93	92	92	93	92	93	1	92	93	1
89	89	88	89	89	89	0	88	89	1
85	84	84	85	84	85	1	84	85	1
79	79	79	80	79	79	0	79	80	1
78	77	77	78	77	77	0	77	78	1
73	72	72	73	72	72	0	72	73	1
70	70	70	70	70	70	0	70	70	0
65	64	64	65	64	65	1	64	65	1
60	60	60	61	60	60	0	60	61	1
57	56	56	57	56	57	1	56	57	1
53	53	53	53	53	53	0	53	53	0
49	49	49	49	49	49	0	49	49	0
45	45	44	45	45	45	0	44	45	1
37	37	37	37	37	37	0	37	37	0
33	33	33	33	33	33	0	33	33	0
30	29	29	30	29	30	1	29	30	1
25	25	24	25	25	25	0	24	25	1
21	21	21	21	21	21	0	21	21	0
17	17	17	17	17	17	0	17	17	0
13	13	13	13	13	13	0	13	13	0
9	9	9	10	9	10	1	9	10	1
5	5	5	5	5	5	0	5	5	0
4	4	4	4	4	4	0	4	4	0
4	4	4	4	4	4	0	4	4	0
3	3	3	3	3	3	0	3	3	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	1	2	2	2	0	1	2	1
1	1	1	1	1	1	0	1	1	0
1	1	1	1	1	1	0	1	1	0
1	1	1	1	1	1	0	1	1	0
0	0	0	0	0	0	0	0	0	0

Table 11-6: The analysis of within measurements for the equipment warm-up reproducibility experiment for unloading before dismantling, transportation and reassembly of the equipment. The analysis relates to 4 repeated measurements at 1-hour interval. All data is in kilo-Pascals.

12.2.3 Summary of equipment warm-up reproducibility results for loading measurements taken after dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentile 5th	Percentile 95th	90% range
0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	0	1	1	0
1	1	1	1	1	1	0	1	1	0
1	1	1	1	1	1	0	1	1	0
2	2	1	2	2	2	0	1	2	1
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
3	2	2	3	2	2	0	2	3	1
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	3	2	3	3	3	0	2	3	1
4	4	4	4	4	4	0	4	4	0
4	4	4	4	4	4	0	4	4	0
5	5	4	5	5	5	0	4	5	1
9	9	9	9	9	9	0	9	9	0
13	12	12	13	12	12	0	12	13	1
17	17	16	17	17	17	0	16	17	1
20	20	20	21	20	21	1	20	21	1
25	25	25	25	25	25	0	25	25	0
29	29	29	29	29	29	0	29	29	0
33	33	32	33	33	33	0	32	33	1
37	37	37	37	37	37	0	37	37	0
45	44	44	45	44	45	1	44	45	1
50	49	49	50	49	49	0	49	50	1
53	52	52	53	52	53	1	52	53	1
57	57	57	57	57	57	0	57	57	0
60	60	59	60	60	60	0	59	60	1
65	64	64	65	64	65	1	64	65	1
70	70	69	70	70	70	0	69	70	1
72	72	72	73	72	72	0	72	73	1
77	77	77	77	77	77	0	77	77	0
80	79	79	80	79	80	1	79	80	1
85	84	84	85	84	85	1	84	85	1
90	89	89	90	89	89	0	89	90	1
92	92	92	93	92	92	0	92	93	1
96	96	95	96	96	96	0	95	96	1
100	100	99	100	100	100	0	99	100	1

Table 11-7: The analysis of within measurements for the equipment warm-up reproducibility experiment for loading after dismantling, transportation and reassembly of the equipment. The analysis relates to 4 repeated measurements at 1-hour interval. All data is in kilo-Pascals.

12.2.4 Summary of equipment warm-up reproducibility results for unloading measurements after before dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles		IQR	Percentile		90% range
				25th	75th		5th	95th	
100	100	99	100	100	100	0	99	100	1
96	96	95	96	96	96	0	95	96	1
93	92	92	93	92	93	1	92	93	1
89	89	89	89	89	89	0	89	89	0
85	85	84	85	85	85	0	84	85	1
79	79	79	79	79	79	0	79	79	0
78	77	77	78	77	78	1	77	78	1
73	72	71	73	72	72	0	71	73	2
70	70	69	70	70	70	0	69	70	1
65	65	64	65	65	65	0	64	65	1
60	60	60	61	60	60	0	60	61	1
56	56	56	57	56	56	0	56	57	1
53	53	53	53	53	53	0	53	53	0
49	49	48	49	49	49	0	48	49	1
44	44	44	45	44	44	0	44	45	1
37	37	36	37	37	37	0	36	37	1
33	33	32	33	33	33	0	32	33	1
30	30	29	30	30	30	0	29	30	1
25	25	25	25	25	25	0	25	25	0
21	21	20	21	21	21	0	20	21	1
17	17	17	17	17	17	0	17	17	0
12	12	12	13	12	12	0	12	13	1
8	8	8	9	8	9	1	8	9	1
5	4	4	5	4	5	1	4	5	1
4	4	4	4	4	4	0	4	4	0
4	4	3	4	4	4	0	3	4	1
3	3	3	3	3	3	0	3	3	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
1	1	1	1	1	1	0	1	1	0
1	1	1	1	1	1	0	1	1	0
1	1	1	1	1	1	0	1	1	0
0	0	0	0	0	0	0	0	0	0

Table 11-8: The analysis of within measurements for the equipment warm-up reproducibility experiment for unloading after dismantling, transportation and reassembly of the equipment. The analysis relates to 4 repeated measurements at 1-hour interval. All data is in kilo-Pascals.

12.3 The within measurements analysis of day-to-day reproducibility for the strain gauge.

12.3.1 Summary of equipment day to day reproducibility results for loading measurements taken before dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles		IQR	Percentile		90% range
				25th	75th		5th	95th	
0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	0	1	1	0
1	1	1	1	1	1	0	1	1	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
3	3	3	3	3	3	0	3	3	0
4	4	4	4	4	4	0	4	4	0
5	5	4	5	5	5	0	4	5	1
5	5	5	5	5	5	0	5	5	0
10	10	10	10	10	10	0	10	10	0
13	13	13	13	13	13	0	13	13	0
18	18	17	18	18	18	0	17	18	1
22	22	21	22	22	22	0	21	22	1
25	26	25	26	25	26	1	25	26	1
30	30	30	30	30	30	0	30	30	0
33	33	33	34	33	33	0	33	34	1
37	38	37	38	37	38	1	37	38	1
45	45	45	45	45	45	0	45	45	0
49	50	49	50	50	50	0	49	50	1
52	54	52	54	54	54	0	52	54	2
57	57	56	57	57	57	0	56	57	1
59	60	59	61	60	61	1	59	61	2
66	66	65	66	66	66	0	65	66	1
69	70	69	71	70	71	1	69	71	2
72	73	72	74	73	74	1	72	74	2
76	77	76	79	76	78	2	76	79	3
78	80	78	81	79	80	1	78	81	3
83	85	83	86	84	86	2	83	86	3
87	89	87	90	88	90	2	87	90	3
92	92	92	94	92	94	2	92	94	2
94	96	94	97	94	97	3	94	97	3
100	100	98	101	100	100	0	98	101	2

Table 11-9: The analysis of within measurements for the day-to-day reproducibility experiment for loading before dismantling, transportation and reassembly of the equipment. The analysis relates to 5 repeated measurements taken over 5 different days. All data is in kilo-Pascals.

12.3.2 Summary of day to day reproducibility results for unloading measurements taken before dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles		IQR	Percentile		90% range
				25th	75th		5th	95th	
99	100	99	101	100	100	0	99	101	2
97	96	94	97	95	97	2	94	97	3
92	92	92	94	92	92	0	92	94	2
90	90	87	90	88	90	2	87	90	3
85	85	83	86	84	85	1	83	86	3
79	79	78	80	79	80	1	78	80	2
79	76	76	79	76	77	1	76	79	3
72	73	72	74	72	73	1	72	74	2
71	70	70	71	70	71	1	70	71	1
65	65	65	66	65	66	1	65	66	1
61	61	60	61	60	61	1	60	61	1
57	57	57	57	57	57	0	57	57	0
54	54	52	54	52	54	2	52	54	2
51	50	49	51	50	50	0	49	51	2
46	46	45	46	46	46	0	45	46	1
38	37	37	38	37	38	1	37	38	1
33	33	33	33	33	33	0	33	33	0
30	30	29	30	30	30	0	29	30	1
26	26	25	26	25	26	1	25	26	1
22	22	22	22	22	22	0	22	22	0
17	17	17	18	17	18	1	17	18	1
13	13	13	13	13	13	0	13	13	0
10	10	9	10	10	10	0	9	10	1
5	5	5	5	5	5	0	5	5	0
5	5	5	5	5	5	0	5	5	0
4	4	4	4	4	4	0	4	4	0
3	3	3	3	3	3	0	3	3	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
1	1	1	2	1	2	1	1	2	1
1	1	1	1	1	1	0	1	1	0
1	1	1	1	1	1	0	1	1	0
0	0	0	0	0	0	0	0	0	0

Table 11-10: The analysis of within measurements for the day-to-day reproducibility experiment for unloading before dismantling, transportation and reassembly of the equipment. The analysis relates to 5 repeated measurements taken over 5 different days. All data is in kilo-Pascals.

12.3.3 Summary of day to day reproducibility results for loading measurements taken after dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentiles 5th	Percentiles 95th	90% range
0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	0	1	1	0
1	1	1	1	1	1	0	1	1	0
2	2	1	2	2	2	0	1	2	1
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
3	3	2	3	3	3	0	2	3	1
4	4	4	4	4	4	0	4	4	0
4	4	4	5	4	5	1	4	5	1
5	5	5	5	5	5	0	5	5	0
10	10	9	10	10	10	0	9	10	1
12	12	12	13	12	13	1	12	13	1
18	18	18	18	18	18	0	18	18	0
21	22	21	22	21	22	1	21	22	1
25	25	25	26	25	26	1	25	26	1
30	30	29	30	29	30	1	29	30	1
33	33	33	33	33	33	0	33	33	0
37	37	37	38	37	37	0	37	38	1
45	45	44	45	45	45	0	44	45	1
50	50	50	50	50	50	0	50	50	0
54	54	54	54	54	54	0	54	54	0
57	56	56	57	56	57	1	56	57	1
61	61	60	61	60	61	1	60	61	1
66	66	66	66	66	66	0	66	66	0
71	71	69	71	70	71	1	69	71	2
74	73	72	74	72	74	2	72	74	2
77	78	76	79	77	78	1	76	79	3
80	80	78	80	78	80	2	78	80	2
86	85	84	86	84	86	2	84	86	2
90	89	87	90	88	90	2	87	90	3
94	94	94	94	94	94	0	94	94	0
97	96	94	97	94	97	3	94	97	3
100	100	99	100	99	100	1	99	100	1

Table 11-11: The analysis of within measurements for the day-to-day reproducibility experiment for loading after dismantling, transportation and reassembly of the equipment. The analysis relates to 5 repeated measurements taken over 5 different days. All data is in kilo-Pascals.

12.3.4 Summary of day to day reproducibility results for unloading measurements after before dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentile 5th	Percentile 95th	90% range
99	99	99	100	99	100	1	99	100	1
96	96	94	96	95	96	1	94	96	2
92	93	92	94	92	93	1	92	94	2
90	88	87	90	87	90	3	87	90	3
83	85	83	86	83	85	2	83	86	3
79	79	78	79	79	79	0	78	79	1
79	78	76	79	77	79	2	76	79	3
72	72	72	73	72	72	0	72	73	1
71	70	70	71	70	70	0	70	71	1
65	66	65	66	65	66	1	65	66	1
60	60	60	61	60	60	0	60	61	1
57	57	56	57	57	57	0	56	57	1
54	53	52	54	52	53	1	52	54	2
50	50	50	50	50	50	0	50	50	0
46	45	45	46	45	46	1	45	46	1
38	38	37	38	38	38	0	37	38	1
33	32	32	33	32	33	1	32	33	1
30	30	30	30	30	30	0	30	30	0
26	25	24	26	25	26	1	24	26	2
22	22	21	22	22	22	0	21	22	1
17	18	17	18	18	18	0	17	18	1
12	13	12	13	13	13	0	12	13	1
10	10	10	10	10	10	0	10	10	0
5	5	5	5	5	5	0	5	5	0
5	5	4	5	5	5	0	4	5	1
4	4	4	4	4	4	0	4	4	0
3	3	3	3	3	3	0	3	3	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
2	2	2	2	2	2	0	2	2	0
1	1	1	1	1	1	0	1	1	0
1	1	1	1	1	1	0	1	1	0
1	1	1	1	1	1	0	1	1	0
0	0	0	0	0	0	0	0	0	0

Table 11-12: The analysis of within measurements for the day-to-day reproducibility experiment for unloading after dismantling, transportation and reassembly of the equipment. The analysis relates to 5 repeated measurements taken over 5 different days. All data is in kilo-Pascals.

APPENDIX 5

12. APPENDIX 5: Within analysis for Novel pressure system

13.1 The within measurements analysis of repeatability for the Novel pressure system.

13.1.1 Summary of repeatability results for loading measurements taken before dismantling, transportation and reassembly of equipment.

Applied load	Median	Minimum	Maximum	Percentiles		IQR	Percentile		90% range
				25th	75th		5th	95th	
0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.20 (20)	0.21 (21)	0.20 (20)	0.22 (22)	0.20 (20)	0.21 (21)	0.01 (1)	0.20 (20)	0.22 (22)	0.02 (2)
0.40 (40)	0.40 (40)	0.40 (40)	0.41 (41)	0.40 (40)	0.40 (40)	0 (0)	0.40 (40)	0.41 (41)	0.01 (1)
0.60 (60)	0.60 (60)	0.60 (60)	0.61 (61)	0.60 (60)	0.61 (61)	0.01 (1)	0.60 (60)	0.61 (61)	0.01 (1)
0.80 (80)	0.81 (81)	0.80 (80)	0.81 (81)	0.80 (80)	0.81 (81)	0.01 (1)	0.80 (80)	0.81 (81)	0.01 (1)
1.00 (100)	1.00 (100)	1.00 (100)	1.01 (101)	1.00 (100)	1.00 (100)	0 (0)	1.00 (100)	1.01 (101)	0.01 (1)
1.20 (120)	1.22 (122)	1.20 (120)	1.22 (122)	1.22 (122)	1.22 (122)	0 (0)	1.20 (120)	1.22 (122)	0.02 (2)
1.40 (140)	1.40 (140)	1.40 (140)	1.40 (140)	1.40 (140)	1.40 (140)	0 (0)	1.40 (140)	1.40 (140)	00 (0)
1.60 (160)	1.60 (160)	1.60 (160)	1.61 (161)	1.60 (160)	1.61 (161)	0.01 (1)	1.60 (160)	1.61 (161)	0.01 (1)
1.80 (180)	1.80 (180)	1.80 (180)	1.83 (183)	1.80 (180)	1.80 (180)	0 (0)	1.80 (180)	1.82 (182)	0.02 (2)
2.00 (200)	2.02 (202)	2.00 (200)	2.02 (202)	2.02 (202)	2.02 (202)	0 (0)	2.00 (200)	2.02 (202)	0.02 (2)

Table 12-1: The analysis of within measurements for the repeatability experiment for loading before dismantling, transportation and reassembly of the equipment. The analysis relates to 5 repeated measurements. All data is in Bar (kilo-Pascals).

13.1.2 Summary of repeatability results for unloading measurements taken before dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentile 5th	Percentile 95th	90% range
2.00 (200)	2.00	2.00	2.01	2.00	2.01	0.01 (1)	2.00	2.01	0.01 (1)
1.80 (180)	1.80	1.80	1.81	1.80	1.80	0 (0)	1.80	1.81	0.01 (1)
1.60 (160)	1.60	1.60	1.61	1.60	1.60	0 (0)	1.60	1.61	0.01 (1)
1.40 (140)	1.40	1.40	1.41	1.40	1.41	0.01 (1)	1.40	1.41	0.01 (1)
1.20 (120)	1.20	1.20	1.22	1.20	1.21	0.01 (1)	1.20	1.22	0.02 (2)
1.00 (100)	1.01	1.00	1.01	1.00	1.01	0.01 (1)	1.00	1.01	0.01 (1)
0.80 (80)	0.80	0.80	0.81	0.80	0.80	0 (0)	0.80	0.81	0.01 (1)
0.60 (60)	0.61	0.60	0.61	0.60	0.61	0.01 (1)	0.60	0.61	0.01 (1)
0.40 (40)	0.40	0.40	0.42	0.40	0.41	0.01 (1)	0.40	0.42	0.02 (2)
0.20 (20)	0.20	0.20	0.21	0.20	0.21	0.01 (1)	0.20	0.21	0.01 (1)
0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 12-2: The analysis of within measurements for the repeatability experiment for unloading before dismantling, transportation and reassembly of the equipment. The analysis relates to 5 repeated measurements. All data is in Bar (kilo-Pascals).

13.1.3 Summary of repeatability results for loading measurements taken after dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentile 5th	Percentile 95th	90% range
0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.20 (20)	0.20 (20)	0.20 (20)	0.21 (21)	0.20 (20)	0.20 (20)	0 (0)	0.20 (20)	0.21 (21)	0.01 (1)
0.40 (40)	0.41 (41)	0.40 (40)	0.41 (41)	0.40 (40)	0.41 (41)	0.01 (1)	0.40 (40)	0.41 (41)	0.01 (1)
0.60 (60)	0.60 (60)	0.60 (60)	0.61 (61)	0.60 (60)	0.61 (61)	0.01 (1)	0.60 (60)	0.61 (61)	0.01 (1)
0.80 (80)	0.80 (80)	0.80 (80)	0.81 (81)	0.80 (80)	0.80 (80)	0 (0)	0.80 (80)	0.81 (81)	0.01 (1)
1.00 (100)	1.00 (100)	1.00 (100)	1.01 (101)	1.00 (100)	1.01 (101)	0.01 (1)	1.00 (100)	1.01 (101)	0.01 (1)
1.20 (120)	1.20 (120)	1.20 (120)	1.21 (121)	1.20 (120)	1.20 (120)	0 (0)	1.20 (120)	1.21 (121_	0.01 (1)
1.40 (140)	1.41 (141)	1.40 (140)	1.41 (141)	1.40 (140)	1.41 (141)	0.01 (1)	1.40 (140)	1.41 (141)	0.01 (1)
1.60 (160)	1.60 (160)	1.60 (160)	1.61 (161)	1.60 (160)	1.60 (160)	0 (0)	1.60 (160)	1.61 (161)	0.01 (1)
1.80 (180)	1.80 (180)	1.80 (180)	1.81 (181)	1.80 (180)	1.80 (180)	0 (0)	1.80 (180)	1.81 (181)	0.01 (1)
2.00 (200)	2.00 (200)	2.00 (200)	2.01 (201)	2.00 (200)	2.00 (200)	0 (0)	2.00 (200)	2.01 (201)	0.01 (1)

Table 12-3: The analysis of within measurements for the repeatability experiment for loading after dismantling, transportation and reassembly of the equipment. The analysis relates to 5 repeated measurements. All data is in Bar (kilo-Pascals).

13.1.4 Summary of repeatability results for unloading measurements after before dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles		IQR	Percentile		90% range
				25th	75th		5th	95th	
2.00 (200)	2.00 (200)	2.00 (200)	2.01 (201)	2.00 (200)	2.00 (200)	0 (0)	2.00 (200)	2.01 (201)	0.01 (1)
1.80 (180)	1.80 (180)	1.80 (180)	1.81 (181)	1.80 (180)	1.81 (181)	0.01 (1)	1.80 (180)	1.81 (181)	0.01 (1)
1.60 (160)	1.61 (161)	1.60 (160)	1.61 (161)	1.60 (160)	1.61 (161)	0.01 (1)	1.60 (160)	1.61 (161)	0.01 (1)
1.40 (140)	1.40 (140)	1.40 (140)	1.41 (141)	1.40 (140)	1.41 (141)	0.01 (1)	1.40 (140)	1.41 (141)	0.01 (1)
1.20 (120)	1.20 (120)	1.20 (120)	1.21 (121)	1.20 (120)	1.21 (121)	0.01 (1)	1.20 (120)	1.21 (121)	0.01 (1)
1.00 (100)	1.00 (100)	1.00 (100)	1.01 (101)	1.00 (100)	1.00 (100)	0 (0)	1.00 (100)	1.01 (101)	0.01 (1)
0.80 (80)	0.80 (80)	0.80 (80)	0.81 (81)	0.80 (80)	0.80 (80)	0 (0)	0.80 (80)	0.81 (81)	0.01 (1)
0.60 (60)	0.60 (60)	0.60 (60)	0.61 (61)	0.60 (60)	0.61 (61)	0.01 (1)	0.60 (60)	0.61 (61)	0.01 (1)
0.40 (40)	0.40 (40)	0.40 (40)	0.41 (41)	0.40 (40)	0.41 (41)	0.01 (1)	0.40 (40)	0.41 (41)	0.01 (1)
0.20 (20)	0.20 (20)	0.20 (20)	0.21 (21)	0.20 (20)	0.20 (20)	0 (0)	0.20 (20)	0.21 (21)	0.01 (1)
0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 12-4: The analysis of within measurements for the repeatability experiment for unloading after dismantling, transportation and reassembly of the equipment. The analysis relates to 5 repeated measurements. All data is in Bar (kilo-Pascals).

13.2 The within measurements analysis of equipment warm-up reproducibility for the Novel pressure system.

13.2.1 Summary of equipment warm-up reproducibility results for loading measurements taken before dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles		IQR	Percentile		90% range
				25th	75th		5th	95th	
0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.20 (20)	0.20 (20)	0.20 (20)	0.21 (21)	0.20 (20)	0.20 (20)	0 (0)	0.20 (20)	0.21 (21)	0.01(1)
0.40 (40)	0.40 (40)	0.40 (40)	0.40 (40)	0.40 (40)	0.40 (40)	0 (0)	0.40 (40)	0.40 (40)	0 (0)
0.60 (60)	0.60 (60)	0.60 (60)	0.60 (60)	0.60 (60)	0.60 (60)	0 (0)	0.60 (60)	0.60 (60)	0 (0)
0.80 (80)	0.80 (80)	0.80 (80)	0.81 (81)	0.80 (80)	0.80 (80)	0 (0)	0.80 (80)	0.81 (81)	0.01(1)
1.00 (100)	1.00 (100)	1.00 (100)	1.00 (100)	1.00 (100)	1.00 (100)	0 (0)	1.00 (100)	1.00 (100)	0 (0)
1.20 (120)	1.20 (120)	1.20 (120)	1.21 (121)	1.20 (120)	1.20 (120)	0 (0)	1.20 (120)	1.21 (121)	0.01(1)
1.40 (140)	1.40 (140)	1.40 (140)	1.40 (140)	1.40 (140)	1.40 (140)	0 (0)	1.40 (140)	1.40 (140)	0 (0)
1.60 (160)	1.60 (160)	1.60 (160)	1.61 (161)	1.60 (160)	1.60 (160)	0 (0)	1.60 (160)	1.61 (161)	0.01(1)
1.80 (180)	1.80 (180)	1.80 (180)	1.81 (181)	1.80 (180)	1.80 (180)	0 (0)	1.80 (180)	1.81 (181)	0.01(1)
2.00 (200)	2.00 (200)	2.00 (200)	2.00 (200)	2.00 (200)	2.00 (200)	0 (0)	2.00 (200)	2.00 (200)	0 (0)

Table 12-5: The analysis of within measurements for the equipment warm-up reproducibility experiment for loading before dismantling, transportation and reassembly of the equipment. The analysis relates to 4 repeated measurements at 1-hour interval. All data is in Bar (kilo-Pascals).

13.2.2 Summary of equipment warm-up reproducibility results for unloading measurements taken before dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentile 5th	Percentile 95th	90% range
2.00 (200)	2.00 (200)	2.00 (200)	2.01 (201)	2.00 (200)	2.00 (200)	0 (0)	2.00 (200)	2.01 (201)	0.01 (1)
1.80 (180)	1.80 (180)	1.80 (180)	1.81 (181)	1.80 (180)	1.80 (180)	0 (0)	1.80 (180)	1.81 (181)	0.01 (1)
1.60 (160)	1.60 (160)	1.60 (160)	1.60 160)	1.60 (160)	1.60 (160)	0 (0)	1.60 (160)	1.60 (160)	0 (0)
1.40 (140)	1.40 (140)	1.40 (140)	1.41 (141)	1.40 (140)	1.40 (140)	0 (0)	1.40 (140)	1.41 (141)	0.01 (1)
1.20 (120)	1.20 (120)	1.20 (120)	1.20 (120)	1.20 (120)	1.20 (120)	0 (0)	1.20 (120)	1.20 (120)	0 (0)
1.00 (100)	1.00 (100)	1.00 (100)	1.01 (101)	1.00 (100)	1.00 (100)	0 (0)	1.00 (100)	1.01 (101)	0.01 (1)
0.80 (80)	0.80 (80)	0.80 (80)	0.81 (81)	0.80 (80)	0.80 (80)	0 (0)	0.80 (80)	0.81 (81)	0.01 (1)
0.60 (60)	0.60 (60)	0.60 (60)	0.61 (61)	0.60 (60)	0.60 (60)	0 (0)	0.60 (60)	0.61 (61)	0.01 (1)
0.40 (40)	0.40 (40)	0.40 (40)	0.40 (40)	0.40 (40)	0.40 (40)	0 (0)	0.40 (40)	0.40 (40)	0 (0)
0.20 (20)	0.20 (20)	0.20 (20)	0.21 (21)	0.20 (20)	0.20 (20)	0 (0)	0.20 (20)	0.21 (21)	0.01 (1)
0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 12-6: The analysis of within measurements for the equipment warm-up reproducibility experiment for unloading before dismantling, transportation and reassembly of the equipment. The analysis relates to 4 repeated measurements at 1-hour interval. All data is in Bar (kilo-Pascals).

13.2.3 Summary of equipment warm-up reproducibility results for loading measurements taken after dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentile 5th	Percentile 95th	90% range
0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.20 (20)	0.20 (20)	0.20 (20)	0.21 (21)	0.20 (20)	0.20 (20)	0 (0)	0.20 (20)	0.21 (21)	0.01 (1)
0.40 (40)	0.40 (40)	0.40 (40)	0.40 (40)	0.40 (40)	0.40 (40)	0 (0)	0.40 (40)	0.40 (40)	0 (0)
0.60 (60)	0.60 (60)	0.60 (60)	0.61 (61)	0.60 (60)	0.60 (60)	0 (0)	0.60 (60)	0.61 (61)	0.01 (1)
0.80 (80)	0.80 (80)	0.80 (80)	0.81 (81)	0.80 (80)	0.80 (80)	0 (0)	0.80 (80)	0.81 (81)	0.01 (1)
1.00 (100)	1.00 (100)	1.00 (100)	1.00 (100)	1.00 (100)	1.00 (100)	0 (0)	1.00 (100)	1.00 (100)	0 (0)
1.20 (120)	1.20 (120)	1.20 (120)	1.20 (120)	1.20 (120)	1.20 (120)	0 (0)	1.20 (120)	1.20 (120)	0 (0)
1.40 (140)	1.40 (140)	1.40 (140)	1.40 (140)	1.40 (140)	1.40 (140)	0 (0)	1.40 (140)	1.40 (140)	0 (0)
1.60 (160)	1.60 (160)	1.60 (160)	1.60 (160)	1.60 (160)	1.60 (160)	0 (0)	1.60 (160)	1.60 (160)	0 (0)
1.80 (180)	1.80 (180)	1.80 (180)	1.81 (181)	1.80 (180)	1.80 (180)	0 (0)	1.80 (180)	1.81 (181)	0.01 (1)
2.00 (200)	2.00 (200)	2.00 (200)	2.00 (200)	2.00 (200)	2.00 (200)	0 (0)	2.00 (200)	2.00 (200)	0 (0)

Table 12-7: The analysis of within measurements for the equipment warm-up reproducibility experiment for loading after dismantling, transportation and reassembly of the equipment. The analysis relates to 4 repeated measurements at 1-hour interval. All data is in Bar (kilo-Pascals).

13.2.4 Summary of equipment warm-up reproducibility results for unloading measurements after before dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentile 5th	Percentile 95th	90% range
2.00 (200)	2.00 (200)	2.00 (200)	2.00 (200)	2.00 (200)	2.00 (200)	0 (0)	2.00 (200)	2.00 (200)	0 (0)
1.80 (180)	1.81 (181)	1.80 (180)	1.81 (181)	1.80 (180)	1.81 (181)	0.01 (1)	1.80 (180)	1.81 (181)	0.01 (1)
1.60 (160)	1.60 (160)	1.60 (160)	1.60 (160)	1.60 (160)	1.60 (160)	0 (0)	1.60 (160)	1.60 (160)	0 (0)
1.40 (140)	1.40 (140)	1.40 (140)	1.40 (140)	1.40 (140)	1.40 (140)	0 (0)	1.40 (140)	1.40 (140)	0 (0)
1.20 (120)	1.20 (120)	1.20 (120)	1.20 (120)	1.20 (120)	1.20 (120)	0 (0)	1.20 (120)	1.20 (120)	0 (0)
1.00 (100)	1.00 (100)	1.00 (100)	1.00 (100)	1.00 (100)	1.00 (100)	0 (0)	1.00 (100)	1.00 (100)	0 (0)
0.80 (80)	0.80 (80)	0.80 (80)	0.81 (81)	0.80 (80)	0.80 (80)	0 (0)	0.80 (80)	0.81 (81)	0.01 (1)
0.60 (60)	0.60 (60)	0.60 (60)	0.61 (61)	0.60 (60)	0.60 (60)	0 (0)	0.60 (60)	0.61 (61)	0.01 (1)
0.40 (40)	0.40 (40)	0.40 (40)	0.40 (40)	0.40 (40)	0.40 (40)	0 (0)	0.40 (40)	0.40 (40)	0 (0)
0.20 (20)	0.20 (20)	0.20 (20)	0.20 (20)	0.20 (20)	0.20 (20)	0 (0)	0.20 (20)	0.20 (20)	0 (0)
0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 12-8: The analysis of within measurements for the equipment warm-up reproducibility experiment for unloading after dismantling, transportation and reassembly of the equipment. The analysis relates to 4 repeated measurements at 1-hour interval. All data is in Bar (kilo-Pascals).

13.3 The within measurements analysis of day-to-day reproducibility for the Novel pressure system.

13.3.1 Summary of equipment day to day reproducibility results for loading measurements taken before dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles		IQR	Percentile		90% range
				25th	75th		5th	95th	
0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.20 (20)	0.21 (21)	0.20 (20)	0.22 (22)	0.20 (20)	0.20 (20)	0 (0)	0.21 (21)	0.22 (22)	0.02 (2)
0.40 (40)	0.40 (40)	0.40 (40)	0.40 (40)	0.40 (40)	0.40 (40)	0 (0)	0.40 (40)	0.40 (40)	0 (0)
0.60 (60)	0.60 (60)	0.60 (60)	0.61 (61)	0.60 (60)	0.60 (60)	0 (0)	0.61 (61)	0.61 (61)	0.01 (1)
0.80 (80)	0.80 (80)	0.80 (80)	0.81 (81)	0.80 (80)	0.80 (80)	0 (0)	0.80 (80)	0.81 (81)	0.01 (1)
1.00 (100)	1.00 (100)	1.00 (100)	1.01 (101)	1.00 (100)	1.00 (100)	0 (0)	1.00 (100)	1.01 (101)	0.01 (1)
1.20 (120)	1.20 (120)	1.20 (120)	1.21 (121)	1.20 (120)	1.20 (120)	0 (0)	1.20 (120)	1.21 (121)	0.01 (1)
1.40 (140)	1.40 (140)	1.40 (140)	1.42 (142)	1.40 (140)	1.40 (140)	0 (0)	1.40 (140)	1.42 (142)	0.02 (2)
1.60 (160)	1.60 (160)	1.60 (160)	1.61 (161)	1.60 (160)	1.60 (160)	0 (0)	1.61 (161)	1.61 (161)	0.01 (1)
1.80 (180)	1.80 (180)	1.80 (180)	1.81 (181)	1.80 (180)	1.80 (180)	0 (0)	1.80 (180)	1.81 (181)	0.01 (1)
2.00 (200)	2.00 (200)	2.00 (200)	2.01 (201)	2.00 (200)	2.00 (200)	0 (0)	2.00 (200)	2.01 (201)	0.01 (1)

Table 12-9: The analysis of within measurements for the day-to-day reproducibility experiment for loading before dismantling, transportation and reassembly of the equipment. The analysis relates to 5 repeated measurements taken over 5 different days. All data is in Bar (kilo-Pascals).

13.3.2 Summary of day to day reproducibility results for unloading measurements taken before dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentile 5th	Percentile 95th	90% range
2.00 (200)	2.00 (200)	2.00 (200)	2.01 (201)	2.00 (200)	2.00 (200)	0 (0)	2.00 (200)	2.01 (201)	0.01 (1)
1.80 (180)	1.80 (180)	1.80 (180)	1.81 (181)	1.80 (180)	1.80 (180)	0 (0)	1.80 (180)	1.81 (181)	0.01 (1)
1.60 (160)	1.60 (160)	1.60 (160)	1.61 (161)	1.60 (160)	1.61 (161)	0.01 (1)	1.60 (160)	1.61 (161)	0.01 (1)
1.40 (140)	1.40 (140)	1.40 (140)	1.41 (141)	1.40 (140)	1.41 (141)	0.01 (1)	1.40 (140)	1.41 (141)	0.01 (1)
1.20 (120)	1.20 (120)	1.20 (120)	1.21 (121)	1.20 (120)	1.20 (120)	0 (0)	1.20 (120)	1.21 (121)	0.01 (1)
1.00 (100)	1.00 (100)	1.00 (100)	1.03 (103)	1.00 (100)	1.00 (100)	0 (0)	1.00 (100)	1.02 (102)	0.02 (2)
0.80 (80)	0.80 (80)	0.80 (80)	0.82 (82)	0.80 (80)	0.81 (81)	0.01 (1)	0.80 (80)	0.82 (82)	0.02 (2)
0.60 (60)	0.60 (60)	0.60 (60)	0.61 (61)	0.60 (60)	0.61 (61)	0.01 (1)	0.60 (60)	0.61 (61)	0.01 (1)
0.40 (40)	0.40 (40)	0.40 (40)	0.41 (41)	0.40 (40)	0.40 (40)	0 (0)	0.40 (40)	0.41 (41)	0.01 (1)
0.20 (20)	0.20 (20)	0.20 (20)	0.21 (21)	0.20 (20)	0.20 (20)	0 (0)	0.20 (20)	0.21 (21)	0.01 (1)
0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 12-10: The analysis of within measurements for the day-to-day reproducibility experiment for unloading before dismantling, transportation and reassembly of the equipment. The analysis relates to 5 repeated measurements taken over 5 different days. All data is in Bar (kilo-Pascals).

13.3.3 Summary of day to day reproducibility results for loading measurements taken after dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles 25th	Percentiles 75th	IQR	Percentile 5th	Percentile 95th	90% range
0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.20 (20)	0.21 (21)	0.20 (20)	0.21 (21)	0.20 (20)	0.21 (21)	0.01 (1)	0.20 (20)	0.21 (21)	0.01 (1)
0.40 (40)	0.40 (40)	0.40 (40)	0.41 (41)	0.40 (40)	0.41 (41)	0.01 (1)	0.40 (40)	0.41 (41)	0.01 (1)
0.60 (60)	0.61 (61)	0.60 (60)	0.61 (61)	0.61 (61)	0.61 (61)	0.01 (1)	0.60 (60)	0.61 (61)	0.01 (1)
0.80 (80)	0.81 (81)	0.81 (81)	0.84 (84)	0.81 (81)	0.81 (81)	0.01 (1)	0.81 (81)	0.83 (83)	0.03 (3)
1.00 (100)	1.00 (100)	1.00 (100)	1.01 (101)	1.00 (100)	1.00 (100)	0 (0)	1.00 (100)	1.01 (101)	0.01 (1)
1.20 (120)	1.20 (120)	1.20 (120)	1.22 (122)	1.20 (120)	1.20 (120)	0 (0)	1.20 (120)	1.22 (122)	0.02 (2)
1.40 (140)	1.40 (140)	1.40 (140)	1.41 (141)	1.40 (140)	1.40 (140)	0 (0)	1.40 (140)	1.41 (141)	0.01 (1)
1.60 (160)	1.60 (1600	1.60 (160)	1.62 (162)	1.60 (160)	1.61 (161)	0.01 (1)	1.60 (160)	1.62 (162)	0.02 (2)
1.80 (180)	1.80 (180)	1.80 (180)	1.81 (181)	1.80 (180)	1.80 (180)	0 (0)	1.80 (180)	1.81 (181)	0.01 (1)
2.00 (200)	2.00 (200)	2.00 (200)	2.02 (202)	2.00 (200)	2.00 (200)	0 (0)	2.00 (200)	2.00 (202)	0.02 (2)

Table 12-11: The analysis of within measurements for the day-to-day reproducibility experiment for loading after dismantling, transportation and reassembly of the equipment. The analysis relates to 5 repeated measurements taken over 5 different days. All data is in Bar (kilo-Pascals).

13.3.4 Summary of day to day reproducibility results for unloading measurements after before dismantling, transportation and reassembly of equipment

Applied load	Median	Minimum	Maximum	Percentiles		IQR	Percentile		90% range
				25th	75th		5th	95th	
2.00 (200)	2.00 (200)	2.00 (200)	2.00 (200)	2.00 (200)	2.00 (200)	0 (0)	2.00 (200)	2.00 (200)	0 (0)
1.80 (180)	1.80 (180)	1.80 (180)	1.80 (180)	1.80 (180)	1.80 (180)	0 (0)	1.80 (180)	1.80 (180)	0 (0)
1.60 (160)	1.60 (160)	1.60 (160)	1.61 (161)	1.60 (160)	1.60 (160)	0 (0)	1.60 (160)	1.61 (161)	0.01 (1)
1.40 (140)	1.40 (140)	1.40 (140)	1.41 (141)	1.40 (140)	1.40 (140)	0 (0)	1.40 (140)	1.41 (141)	0.01 (1)
1.20 (120)	1.20 (120)	1.20 (120)	1.21 (121)	1.20 (120)	1.20 (120)	0 (0)	1.20 (120)	1.21 (121)	0.01 (1)
1.00 (100)	1.00 (100)	1.00 (100)	1.01 (101)	1.00 (100)	1.00 (100)	0 (0)	1.00 (100)	1.01 (101)	0.01 (1)
0.80 (80)	0.80 (80)	0.80 (80)	0.81 (81)	0.80 (80)	0.80 (80)	0 (0)	0.80 (80)	0.81 (81)	0.01 (1)
0.60 (60)	0.60 (60)	0.60 (60)	0.61 (61)	0.60 (60)	0.60 (60)	0 (0)	0.60 (60)	0.61 (61)	0.01 (1)
0.40 (40)	0.40 (40)	0.40 (40)	0.41 (41)	0.40 (40)	0.40 (40)	0 (0)	0.40 (40)	0.41 (41)	0.01 (1)
0.20 (20)	0.20 (20)	0.20 (20)	0.21 (21)	0.20 (20)	0.21 (21)	0.01 (1)	0.20 (20)	0.21 (21)	0.01 (1)
0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 12-12: The analysis of within measurements for the day-to-day reproducibility experiment for unloading after dismantling, transportation and reassembly of the equipment. The analysis relates to 5 repeated measurements taken over 5 different days. All data is in Bar (kilo-Pascals).

APPENDIX 6

13. APPENDIX 6: Within analysis for laser Doppler Fluxmeter

14.1 The within measurements analysis of repeatability for laser Doppler fluxmeter flux taken on the same day.

14.1.1 Summary of repeatability results for laser Doppler fluxmeter flux at baseline.

Number of repeated measurements	Median	Minimum	Maximum	Percentiles		IQR	Percentiles		90% range
				25th	75th		5th	95th	
2	58.9	29.4	88.4	44.2	73.7	29.5	32.4	85.5	53.1
2	161.5	155.2	167.7	158.3	164.6	6.3	155.8	167.1	11.3
2	66.5	64.7	68.3	65.6	67.4	1.8	64.9	68.1	3.2
2	8.6	8.2	9.0	8.4	8.8	0.4	8.2	9.0	0.7
2	34.3	17.2	51.4	25.8	42.9	17.1	18.9	49.7	30.8
2	10.8	3.7	17.9	7.3	14.4	7.1	4.4	17.2	12.8
2	36.7	26.2	47.2	31.5	42.0	10.5	27.3	46.2	18.9
2	25.6	23.4	27.7	24.5	26.6	2.2	23.6	27.5	3.9
2	55.5	47.2	63.7	51.3	59.6	8.3	48.0	62.9	14.9
2	94.4	85.4	103.3	89.9	98.8	9.0	86.3	102.4	16.1
2	34.7	24.8	44.5	29.7	39.6	9.9	25.8	43.5	17.7
2	153.8	134.1	173.5	144.0	163.7	19.7	136.1	171.5	35.5
2	16.5	13.0	20.0	14.8	18.3	3.5	13.4	19.7	6.3
2	44.5	40.1	48.9	42.3	46.7	4.4	40.5	48.5	7.9

Table 13-1: The summary of baseline results for 2 repeated measures taken on the same day for the between group analysis. All laser Doppler fluxmeter flux values are in Arbitrary Units.

14.1.2 Summary of repeatability results for laser Doppler fluxmeter flux at baseline.

Number of repeated measurements	Median	Minimum	Maximum	Percentiles		IQR	Percentiles		90% range
				25th	75th		5th	95th	
2	11.9	10.1	13.6	11.0	12.7	1.8	10.3	13.4	3.2
2	12.7	9.6	15.7	11.1	14.2	3.1	9.9	15.4	5.5
2	6.8	6.4	7.1	6.6	6.9	0.4	6.4	7.1	0.6
2	3.5	3.5	3.5	3.5	3.5	0.0	3.5	3.5	0.0
2	9.1	8.3	9.8	8.7	9.4	0.8	8.4	9.7	1.4
2	4.1	3.4	4.8	3.8	4.5	0.7	3.5	4.7	1.3
2	8.8	7.7	9.8	8.2	9.3	1.1	7.8	9.7	1.9
2	6.5	5.9	7.1	6.2	6.8	0.6	6.0	7.0	1.1
2	5.8	5.4	6.2	5.6	6.0	0.4	5.4	6.2	0.7
2	20.1	18.8	21.4	19.5	20.8	1.3	18.9	21.3	2.3
2	12.9	12.1	13.6	12.5	13.2	0.8	12.2	13.5	1.4
2	13.6	12.8	14.4	13.2	14.0	0.8	12.9	14.3	1.4
2	14.2	11.8	16.6	13.0	15.4	2.4	12.0	16.4	4.3
2	8.3	8.0	8.5	8.1	8.4	0.3	8.0	8.5	0.4

Table 13-2: The summary of the effects of applying 90 kPa of plantar foot pressure on the skin for 2 repeated measures taken on the same day for the between group analysis. All laser Doppler fluxmeter flux values are in Arbitrary Units.

14.1.3 Summary of repeatability results for laser Doppler fluxmeter flux at baseline.

Number of repeated measurements	Median	Minimum	Maximum	Percentiles		IQR	Percentiles		90% range
				25th	75th		5th	95th	
2	610.5	543.9	677.0	577.2	643.7	66.6	550.6	670.3	119.8
2	783.7	740.0	827.4	761.9	805.6	43.7	744.4	823.0	78.7
2	502.9	452.6	553.1	477.7	528.0	50.3	457.6	548.1	90.5
2	316.4	233.0	399.8	274.7	358.1	83.4	241.3	391.5	150.1
2	499.2	487.7	510.6	493.4	504.9	11.5	488.8	509.5	20.6
2	437.0	421.8	452.1	429.4	444.5	15.2	423.3	450.6	27.3
2	399.1	384.5	413.6	391.8	406.3	14.6	386.0	412.1	26.2
2	886.3	820.0	952.5	853.1	919.4	66.3	826.6	945.9	119.3
2	382.6	304.2	461.0	343.4	421.8	78.4	312.0	453.2	141.1
2	885.5	860.7	910.2	873.1	897.8	24.8	863.2	907.7	44.6
2	665.7	664.5	666.9	665.1	666.3	1.2	664.6	666.8	2.2
2	849.6	815.6	883.6	832.6	866.6	34.0	819.0	880.2	61.2
2	651.4	649.4	653.4	650.4	652.4	2.0	649.6	653.2	3.6
2	660.2	614.8	705.5	637.5	682.8	45.4	619.3	701.0	81.6

Table 13-3: The summary of the hyperaemic response according after applying 90 kPa of plantar foot pressure on the skin for 2 repeated measures taken on the same day for the between group analysis. All laser Doppler fluxmeter flux values are in Arbitrary Units.

14.2 The within measurements analysis of the effects of *in vivo* day-to-day reproducibility of results for laser Doppler fluxmeter flux.

14.2.1 Summary of the effects of *in vivo* day-to-day reproducibility of flux at baseline.

Number of repeated measurements	Median	Minimum	Maximum	Percentiles		IQR	Percentiles		90% range
				25th	75th		5th	95th	
2	17.6	5.8	29.4	11.7	23.5	11.8	7.0	28.2	21.2
2	167.7	155.2	180.1	161.4	173.9	12.5	156.4	178.9	22.4
2	56.7	38.4	75.0	47.6	65.9	18.3	40.2	73.2	32.9
2	45.0	40.5	49.5	42.8	47.3	4.5	41.0	49.1	8.1
2	20.9	15.3	26.5	18.1	23.7	5.6	15.9	25.9	10.1
2	37.1	27.8	46.3	32.4	41.7	9.3	28.7	45.4	16.7
2	29.3	14.0	44.5	21.6	36.9	15.3	15.5	43.0	27.5
2	171.9	86.2	257.5	129.0	214.7	85.7	94.8	248.9	154.2
2	56.5	13.0	99.9	34.7	78.2	43.5	17.3	95.6	78.2
2	73.3	48.9	97.7	61.1	85.5	24.4	51.3	95.3	43.9

Table 13-4: The summary of baseline results for 2 repeated measures taken on 2 different days for the between group analysis. All laser Doppler fluxmeter flux values are in Arbitrary Units.

14.2.2 Summary of the effects of *in vivo* day-to-day reproducibility of flux after the application of 90 kPa of plantar foot pressure on the skin.

Number of repeated measurements	Median	Minimum	Maximum	Percentiles		IQR	Percentiles		90% range
				25th	75th		5th	95th	
2	7.0	3.8	10.1	5.4	8.5	3.2	4.1	9.8	5.7
2	9.4	9.2	9.6	9.3	9.5	0.2	9.2	9.6	0.4
2	7.2	5.6	8.8	6.4	8.0	1.6	5.8	8.6	2.9
2	7.2	5.2	9.2	6.2	8.2	2.0	5.4	9.0	3.6
2	5.4	4.8	6.0	5.1	5.7	0.6	4.9	5.9	1.1
2	9.2	6.9	11.5	8.1	10.4	2.3	7.1	11.3	4.1
2	10.1	6.5	13.6	8.3	11.8	3.6	6.9	13.2	6.4
2	11.4	10.0	12.7	10.7	12.0	1.4	10.1	12.6	2.4
2	12.1	11.8	12.4	12.0	12.3	0.3	11.8	12.4	0.5
2	9.8	8.5	11.0	9.1	10.4	1.3	8.6	10.9	2.3

Table 13-5: The summary of the effects of applying 90 kPa of plantar foot pressure on the skin for 2 repeated measures taken on 2 different days for the between group analysis. All laser Doppler fluxmeter flux values are in Arbitrary Units.

14.2.3 Summary of the effects of *in vivo* day-to-day reproducibility of flux during the hyperaemic response after the plantar foot pressure was removed.

Number of repeated measurements	Median	Minimum	Maximum	Percentiles		IQR	Percentiles		90% range
				25^{th}	75^{th}		5^{th}	95^{th}	
2	565.1	543.9	586.2	554.5	575.6	21.2	546.0	584.1	38.1
2	661.8	583.6	740.0	622.7	700.9	78.2	591.4	732.2	140.8
2	573.0	530.9	615.0	551.9	594.0	42.1	535.1	610.8	75.7
2	575.7	502.1	649.3	538.9	612.5	73.6	509.5	641.9	132.5
2	388.3	304.0	472.6	346.2	430.5	84.3	312.4	464.2	151.7
2	263.4	195.9	330.9	229.7	297.2	67.5	202.7	324.2	121.5
2	642.8	618.7	666.9	630.8	654.9	24.1	621.1	664.5	43.4
2	736.6	662.9	810.2	699.7	773.4	73.7	670.3	802.8	132.6
2	593.2	536.9	649.4	565.0	621.3	56.3	542.5	643.8	101.3
2	593.3	571.8	614.8	582.6	604.1	21.5	574.0	612.7	38.7

Table 13-6: The summary of the hyperaemic response according after applying 90 kPa of plantar foot pressure on the skin for 2 repeated measures taken on 2 different days for the between group analysis. All laser Doppler fluxmeter flux values are in Arbitrary Units.

APPENDIX 7

14. APPENDIX 7: Recording of Room Temperature and Humidity

DATE	SUBJECT STUDY NUMBER	ROOM	
		TEMPERATURE (°C)	HUMIDITY

APPENDIX 8

15. APPENDIX 8: Questionnaire for patients attending the Rheumatology Clinic at the Borders General Hospital

Name:.....

1. Have you had rheumatoid arthritis for more than ten years

   (Please tick one of the boxes below)?

☐ YES ☐ NO

If YES,

Would you like to help us with research by volunteering for a study looking at the effects of walking on skin blood flow (Please read the study information sheet)?

☐ YES ☐ NO ☐ UNDECIDED

Please hand this form to the nurse/consultant, where you may ask for further information.

THANKYOU FOR YOUR HELP

APPENDIX 9

16. APPENDIX 9: Study information sheet for RA subjects

An investigation into the effects of walking pressures in the sole of the foot and how it affects the blood flow of the skin

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

rheumatoid arthritis often affects feet and is an area where more research is needed. The study is part of a research project (a number of research studies that will be carried out over a three year period) looking at foot problems in patients with rheumatoid arthritis. Results from this study will provide new knowledge about how walking/standing pressure on the sole of the foot affects the blood circulation of the skin. The study aims to contribute and strengthen current knowledge by investigating the effects of walking pressure on the skin blood flow in the sole of the foot of patients with rheumatoid arthritis. We would like you to help us by taking part.

Why have I been chosen?

Patients attending the Rheumatology Clinic at the Borders General Hospital have been invited to take part in this study. We require approximately 10 participants with rheumatoid arthritis and 10 participants without rheumatoid arthritis.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

Although the study will take a few months to be completed **you only have to attend once.** You will be asked to attend the Borders General hospital once for the study and be asked not to eat or drink anything ½ hour before the study. You will be asked to sit on the couch and asked to place your foot in a special shoe with computerised sensors capable of counting the number of cells that move past the skin. The shoe will apply graduated pressures (equivalent to walking or standing) and the computerised sensors will provide measurements of the skin blood flow to a computer. The study will be repeated with yourself in a sitting position. This should only take about 1½ hours of your time. Travelling expenses will be reimbursed.

What do I have to do?

The only thing that you have to do is not to eat or drink anything half an hour before the study. Do everything else as normal.

What are the possible disadvantages and risks of taking part?

The study only involves the recording of skin blood flow under various walking pressures and thus, there are no possible disadvantages and risks of taking part are minimal.

What are the possible benefits of taking part?

The information we get from this study will allow us to compare if any differences exist between people with rheumatoid arthritis and those without, and help us understand what is happening to the blood flow of the skin when people walk. These finding are clinically important since we can then predict to what level we must reduce pressures on the sole of the foot using insoles to prevent foot problems.

What if something goes wrong?

If taking part in this research project harms you, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms may be available to you. In addition to the above procedure, Queen Margaret University College has a liability insurance scheme for compensation as a result of harm caused due to negligence on the part of the researcher in connection with the mentioned research study.

Will taking part in this study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you, which leaves the hospital, will have your name and address removed so that you cannot be recognised.

What will happen to the results of the study?

Publication of research results usually takes several months once the study has been completed. The results will be published as a research paper in medical journals and will also be available at Queen Margaret University College library. None of the participants will be identified in any reports or medical publications and all data collected will only be kept as long as necessary.

Who is organising and funding the research?

This study is being conducted by Derek Santos, researcher for this study, based at Queen Margaret University College in Edinburgh. The study is funded by Queen Margaret University College as part of a PhD research degree. The study is being supervised by: Dr Thomas Carline, lecturer from Queen Margaret University College; Dr. Rammi Abboud, from the Department of Orthopaedic and Trauma Surgery at the Medical School of the University of Dundee; and Dr Ruth Richmond, Consultant Rheumatologist from NHS Borders.

Who has review this study?

The study has been reviewed by the Borders Research Ethics Committee; Faculty of Health Sciences, Queen Margaret University College; Department of Orthopaedic and Trauma Surgery, Medical School, University of Dundee; Rheumatology Department, NHS Borders.

CONTACT FOR FURTHER INFORMATION

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Note: you will be given a copy of this information sheet and a signed consent form to keep.

THANKYOU FOR TAKING PART IN THIS STUDY.

APPENDIX 10

17. APPENDIX 10: Study information sheet for control subjects

An investigation into the effects of walking pressures in the sole of the foot and how it affects the blood flow of the skin

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

rheumatoid arthritis often affects feet and is an area where more research is needed. The study is part of a research project (a number of research studies that will be carried out over a three year period) looking at foot problems in patients with rheumatoid arthritis. Results from this study will provide new knowledge about how walking/standing pressure on the sole of the foot affects the blood circulation of the skin. The study aims to contribute and strengthen current knowledge by investigating the effects of walking pressure on the skin blood flow in the sole of the foot of patients with rheumatoid arthritis. We would like you to help us by taking part.

Why have I been chosen?

Healthy participants have been invited to take part in this study to allow comparisons against patients with rheumatoid arthritis. We require approximately 10 participants with rheumatoid arthritis and 10 healthy participants without rheumatoid arthritis.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect your rights.

What will happen to me if I take part?

Although the study will take a few months to be completed **you only have to attend once.** You will be asked to attend the Queen Margaret University College (Leith) once for the study and be asked not to eat or drink anything ½ hour before the study. You will be asked to sit on the couch and asked to place your foot in a special shoe with computerised sensors capable of counting the number of cells that move past the skin. The shoe will apply graduated pressures (equivalent to walking or standing) and the computerised sensors will provide measurements of the skin blood flow to a computer. This should only take about 1½ hours of your time. Travelling expenses will be reimbursed.

What do I have to do?

The only thing that you have to do is not to eat or drink anything half an hour before the study. Do everything else as normal.

What are the possible disadvantages and risks of taking part?

The study only involves the recording of skin blood flow under various walking pressures and thus, there are no possible disadvantages and risks of taking part are minimal.

What are the possible benefits of taking part?

The information we get from this study will allow us to compare if any differences exist between people with rheumatoid arthritis and those without, and help us understand what is happening to the blood flow of the skin when people walk. These finding are clinically important since we can then predict to what level we must reduce pressures on the sole of the foot using insoles to prevent foot problems.

What if something goes wrong?

If taking part in this research project harms you, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, Queen Margaret University College has a complaints procedure and a liability insurance scheme for compensation as a result of harm caused due to negligence on the part of the researcher in connection with the mentioned research study.

Will taking part in this study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you, which leaves the University, will have your name and address removed so that you cannot be recognised.

What will happen to the results of the study?

Publication of research results usually takes several months once the study has been completed. The results will be published as a research paper in medical journals and will also be available at Queen Margaret University College library. None of the participants will be identified in any reports or medical publications and all data collected will only be kept as long as necessary.

Who is organising and funding the research?

This study is being conducted by Derek Santos, researcher for this study, based at Queen Margaret University College in Edinburgh. The study is funded by Queen Margaret University College as part of a PhD research degree. The study is being supervised by: Dr Thomas Carline, lecturer from Queen Margaret University College; Dr. Rammi Abboud, from the Department of Orthopaedic and Trauma Surgery at the Medical School of the University of Dundee; and Dr Ruth Richmond, Consultant Rheumatologist from NHS Borders.

Who has review this study?

The study has been reviewed by the Borders Research Ethics Committee; Faculty of Health Sciences, Queen Margaret University College; Department of Orthopaedic and Trauma Surgery, Medical School, University of Dundee; Rheumatology Department, NHS Borders.

CONTACT FOR FURTHER INFORMATION

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Note: you will be given a copy of this information sheet and a signed consent form to keep.

THANKYOU FOR TAKING PART IN THIS STUDY.

APPENDIX 11

18. APPENDIX 11: rheumatoid arthritis and controls data capture sheets

a. rheumatoid arthritis patients data capture sheet

Study Number: ____

rheumatoid arthritis Group

Consent form signed:	□ YES □ NO	Height:		cm
Date of birth:	/ /	Age:		years
Gender:	MALE / FEMALE	Weight:		kg

DRUG HISTORY	OTHER DATA	Left	Right
	Feet size		
	Shoe size		

STUDY CRITERIA

	Inclusion Criteria	Exclusion Criteria
Current smoker?	□ NO	□ YES
Current foot/leg ulcer(s)?	□ NO	□ YES
Current foot/leg gangrene?	□ NO	□ YES
Raynaud's phenomenon?	□ NO	□ YES
Vasoactive drugs taken at present?	□ NO	□ YES
Callus or scar tissue in plantar aspect of heels?	□ NO	□ YES
Fasted half an hour before study?	□ YES	□ NO

Subject Accepted for study? ACCEPTED / REJECTED

DISEASE HISTORY

rheumatoid arthritis disease duration? ____ years ARA Criteria passed: □ YES □ NO

History of previous plantar heel ulcer(s)? □ YES □ NO ESR: mm

If yes, duration since last plantar heel ulcer? ____ years

Rheumatoid Factor? POSITIVE / NEGATIVE

RANDOMISED SEQUENCE FORCE APPLIED

First: kPa Second: kPa Third: kPa

ARA rheumatoid arthritis Criteria	
Criterion	
1) Morning Stiffness9	YES / NO
2) Arthritis of 3 or more joint areas10	YES / NO
3) Arthritis of hand joints11	YES / NO
4) Symmetrical arthritis12	YES / NO
5) Rheumatoid nodules13	YES / NO
6) Serum Rheumatoid Factor14	YES / NO
7) Radiological changes15	YES / NO
SUBJECT MEETS AT LEAST 4 OF ABOVE CRITERIA16	YES / NO

Thinking about your foot pain in the past 4 weeks

Thinking about your foot pain in the last 4 weeks, on the scale below, where "0" is "no pain" and "10" is "worst pain imaginable", draw a vertical line through the point in the scale which represents your foot pain.

0 ——————————————————————— 10

No Worst pain

[9] Morning stiffness in and around the joints, lasting at least 1 hour before maximal improvement, for at least 6 weeks.
[10] At least 3 joint areas simultaneously have had soft tissue swelling or fluid (not bony overgrowth alone) observed by a physician for at least 6 weeks. The 14 possible areas are right or left PIP, MCP, wrist, elbow, knee, ankle, and MTP joints.
[11] At least 1 area swollen (as defined above) in wrist, MCP, or PIP joint, for at least 6 weeks.
[12] Simultaneous involvement of the same joint areas (as defined in 2) on both sides of the body (bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry), for at least 6 weeks.
[13] Subcutaneous nodules, over bony prominences, or extensor surfaces, or in juxtaarticular regions, observed by a physician.
[14] Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in <5% of normal control subjects.
[15] Radiological changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localised in or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify).
[16] For classification purposes, a patient shall be said to have rheumatoid arthritis if he/she has satisfied at least 4 of the above 7 criteria.

b. Healthy controls data capture sheet

Study Number:

Healthy Controls Group

Consent form signed:	□ YES □ NO	Height:		cm
Date of birth:	/ /	Age:		years
Gender:	MALE / FEMALE	Weight :		kg

DRUG HISTORY	**OTHER DATA**	Left	Right
	Feet size		
	Shoe size		

STUDY CRITERIA

	Inclusion Criteria	**Exclusion Criteria**
Current smoker?	□ NO	□ YES
Current or previous foot/leg ulcer(s)?	□ NO	□ YES
Current or previous foot/leg gangrene?	□ NO	□ YES
Raynaud's phenomenon?	□ NO	□ YES
Vasoactive drugs taken at present?	□ NO	□ YES
Callus or scar tissue in plantar aspect of heels?	□ NO	□ YES
Fasted half an hour before study?	□ YES	□ NO

Subject Accepted for study? **ACCEPTED / REJECTED**

RANDOMISED SEQUENCE FORCE APPLIED

First: kPa Second: kPa Third: kPa

c. Patient Demographics Details for Study

STUDY NUMBER	
Surname	First name
Address	
Telephone number	
Mobile number	

7.11.1 STUDY NUMBER	
Surname	First name
Address	
Telephone number	
Mobile number	

7.11.2 STUDY NUMBER	
Surname	First name
Address	
Telephone number	
Mobile number	

This form must be kept in a secure locked place in the Borders General Hospital when not in use by the researcher. The form must not leave the Borders General Hospital. The form will be used for the recording of subject demographic details for the study "An investigation of the effects of plantar foot pressures on the microcirculation of the foot in patients with rheumatoid arthritis" only. At the end of the study the form must be destroyed.
CONFIDENTIAL

PAGE:.....

APPENDIX 12

19. APPENDIX 12: Consent forms

Subject Identification Number for this trial:

RA SUBJECTS CONSENT FORM

Title of Project: **An investigation of the effects of plantar foot pressures on the microcirculation of the foot in patients with rheumatoid arthritis.**

Name of Researcher: Derek Santos
Researcher
Division of Podiatry
Queen Margaret University College
Leith
Edinburgh
EH6 8HF
Tel: 0131 317 3663
E-mail: dsantos@qmuc.ac.uk

Please initial box

☐ 1 I confirm that I have read and understand the information sheet dated ..... (version.. .....) for the above study and have had the opportunity to ask questions.

☐ 2 I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

☐ 3 I understand that sections of any of my medical notes may be looked at by Mr Derek Santos, researcher from Queen Margaret University College or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.

☐ 4 I agree to take part in the above study.

Name of Patient:_____ Date:_____

Signature:_____

Name of Person taking consent:_____ Date:_____

Signature:_____
(If different from researcher)

Researcher:_____ Date:_____

Signature:_____

1 for patient; 1 for researcher; 1 to be kept with hospital notes

Subject Identification Number for this trial:

CONTROL SUBJECTS CONSENT FORM

Title of Project: **An investigation of the effects of plantar foot pressures on the microcirculation of the foot in patients with rheumatoid arthritis.**

Name of Researcher: Derek Santos
Researcher
Division of Podiatry
Queen Margaret University College
Leith
Edinburgh
EH6 8HF
Tel: 0131 317 3663
E-mail: dsantos@qmuc.ac.uk

Please initial box

☐ 1 I confirm that I have read and understand the information sheet dated ..... (version.....) for the above study and have had the opportunity to ask questions.

☐ 2 I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

☐ 3 I understand that sections of any of my medical notes may be looked at by Mr Derek Santos, researcher from Queen Margaret University College or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.

☐ 4 I agree to take part in the above study.

Name of Subject:_____ Date:_____

Signature:_____

Name of Person taking consent:_____ Date:_____

Signature:_____
(If different from researcher)

Researcher:_____ Date:_____

Signature:_____

1 for subject; 1 for researcher

APPENDIX 13

20. APPENDIX 13: Control probe analysis

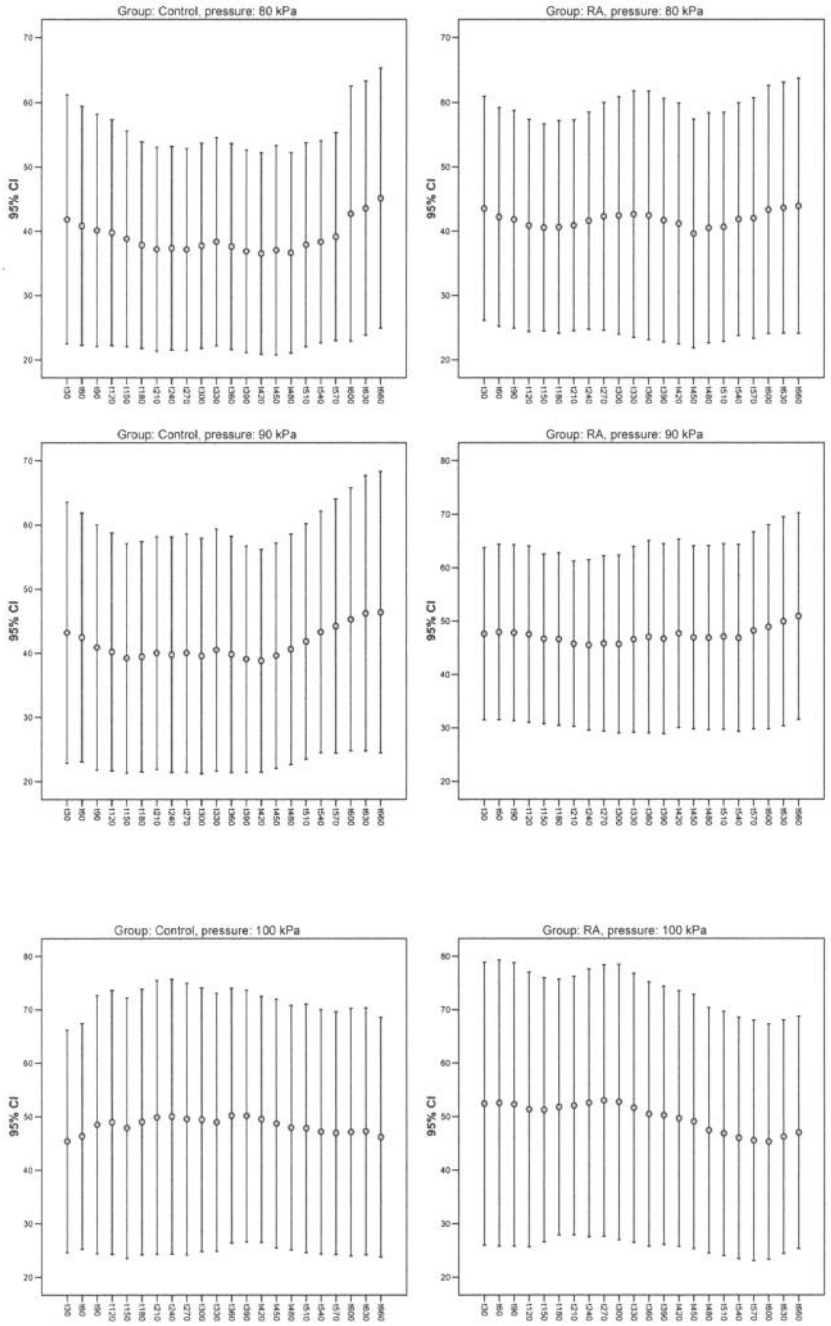

Figure 20-1: Error plots spline graphs at 95% confidence intervals for the control probe that was attached to the plantar aspect of the forefoot within the shoe device. The left charts display the data for the control group and the right for the rheumatoid arthritis group. The top two charts show the data for the application of 80 kPa of pressure to the heel, the middle two of application of 90 kPa of pressure and the bottom two of 100 kPa of pressure.

Time intervals (Seconds)	Applied pressure		
	80 kPa	90 kPa	100 kPa
30	p=0.97	p=0.97	p=0.87
	p>0.05	p>0.05	p>0.05
60	p=1.00	p=0.92	p=0.97
	p>0.05	p>0.05	p>0.05
90	p=0.97	p=0.77	p=0.90
	p>0.05	p>0.05	p>0.05
120	p=0.92	p=0.67	p=0.95
	p>0.05	p>0.05	p>0.05
150	p=0.97	p=0.45	p=0.92
	p>0.05	p>0.05	p>0.05
180	p=0.97	p=0.58	p=0.82
	p>0.05	p>0.05	p>0.05
210	p=0.87	p=0.77	p=0.95
	p>0.05	p>0.05	p>0.05
240	p=0.92	p=0.87	p=0.87
	p>0.05	p>0.05	p>0.05
270	p=0.77	p=0.97	p=0.87
	p>0.05	p>0.05	p>0.05
300	p=0.77	p=0.87	p=0.92
	p>0.05	p>0.05	p>0.05
330	p=0.97	p=0.84	p=0.97
	p>0.05	p>0.05	p>0.05
360	p=0.92	p=0.72	p=0.87
	p>0.05	p>0.05	p>0.05
390	p=0.82	p=0.67	p=0.87
	p>0.05	p>0.05	p>0.05
420	p=0.92	p=0.62	p=0.82
	p>0.05	p>0.05	p>0.05
450	p=1.00	p=0.77	p=0.77
	p>0.05	p>0.05	p>0.05
480	p=0.98	p=0.72	p=0.82
	p>0.05	p>0.05	p>0.05
510	p=0.90	p=0.77	p=0.82
	p>0.05	p>0.05	p>0.05
540	p=0.92	p=0.85	p=0.82
	p>0.05	p>0.05	p>0.05
570	p=0.82	p=0.84	p=0.82
	p>0.05	p>0.05	p>0.05
600	p=0.85	p=0.92	p=0.87
	p>0.05	p>0.05	p>0.05
630	p=0.95	p=0.83	p=0.92
	p>0.05	p>0.05	p>0.05
660	p=0.87	p=0.70	p=0.97
	p>0.05	p>0.05	p>0.05

Table 20-1: Statistical analysis for the comparison between patients with rheunmatoid arthritis and healthy controls for all the data collected with the control probe. The Mann-Whitney U test is shown. None of the results are statistically significant.

Rheumatoid arthritis			Control		
80 kPa	**90 kPa**	**100 kPa**	**80 kPa**	**90 kPa**	**100 kPa**
p=0.87 p>0.05	p=0.88 p>0.05	p=0.06 p>0.05	p=0.75 p>0.05	p=0.34 p>0.05	p=0.84 p>0.05

Table 20-2: Statistical analysis for the within subjects effects for those with rheunmatoid arthritis and healthy controls for all data collected with the control probe. The Friedman's test is shown. None of the results are statistically significant.

APPENDIX 14

21. APPENDIX 14: Publications

22.1 A modular device to measure the effects of plantar foot pressure on the microcirculation of the heel.

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The Foot 13 (2003) 30–38
The Foot
www.elsevierhealth.com/journals/foot

A modular device to measure the effects of plantar foot pressure on the microcirculation of the heel☆

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[b] Rheumatology Department, Borders General Hospital NHS Borders, Melrose, Roxburghshire, Scotland TD6 9BS, UK
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Received 2 April 2002; accepted 17 July 2002

Abstract

Background: Past research has concentrated on foot function and plantar foot pressure, with many devices developed for this purpose. However, little is known of how cutaneous blood flow compensates for ambulatory repetitive circulatory insults and how ulceration occurs.

Objectives: To develop a system to measure the effects of plantar foot pressure on cutaneous blood flow in the supine and semi-weight bearing positions.

Method: A system was developed that integrated a laser Doppler fluxmeter with a pressure probe, allowing plantar foot pressure and skin blood flow to be recorded simultaneously. The system was tested using four volunteers (28 ± 8.6 years).

Results: A significant difference existed between baseline laser Doppler flowmetry (LDF) in the supine and semi-weight bearing positions ($P = 0.023$). Differences between both positions also existed in the reduction in LDF levels following application of pressure ($P = 0.015$), the maximum hyperaemic response ($P = 0.034$) and time taken to reach maximum hyperaemic response ($P = 0.019$).

Conclusion: The device has shown that with current technology it is now possible not only to investigate plantar foot pressure but also how it affects skin blood flow, which in some cases can lead to ulceration. The effect of plantar foot pressure on cutaneous blood flow differs depending on whether the subject is supine or semi-weight bearing. Thus, to understand the effects of plantar foot pressure on skin blood flow future researchers must ensure that subjects are in an upright position when recording.

Keywords: Foot; Microcirculation; Pressure; Skin blood flow; Laser Doppler fluxmeter; Strain gauge

1. Introduction

Human walking involves an upright stance with plantar foot pressure transmitted through the skin onto deeper structures. So far, research has concentrated on the effects of plantar foot pressure on foot function and its postural integrity. Many devices [1] exist that allow us to study plantar foot pressure with little known of how plantar foot pressure interacts or causes damage to soft tissues. An important clinical question that needs to be addressed is whether plantar foot pressure is beneficial or detrimental to cutaneous circulation and tissue viability.

Various methods of investigating the effects of external pressure on the skin's microcirculation have been developed by a number of authors. However, most of the studies do not relate to the foot [2–9]. The few that relate to the foot have been unable to measure the effects of quantifiable plantar foot pressure on the skin's blood flow on the sole of the foot, with the subject in a semi-weight and full-weight bearing positions [10–14] with the exception of Proano et al. [15]. Proano et al. [15] developed a system to measure plantar foot pressure, using the EMED Pressure Analysis System,[1] followed by measuring supine, standing and walking skin blood flow using fluorescein flowmetry. However, one of the limitations of this study was that plantar foot pressure and plantar circulation were not measured simultaneously; subjects were trained to stop on the podoscope and lift the opposite foot for the pictures to be taken. Thus, their measurement

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did not reflect the true walking position but more of a one legged static standing position with measurement taken on the open-kinetic chain foot. Meinders et al. [14] developed a device to measure the microcirculation on the centre of the sole of the heel under quantifiable external pressure with the subject in a supine position using a screw piston with a load cell (HBM load cell, type Z8) and a laser Doppler fluxmeter probe.[2]

In this project, a system using a laser Doppler fluxmeter (DRT4)[3] together with a single strain gauge pressure sensor[4] was synchronised to measure the effects of quantifiable applied plantar foot pressure on skin blood flow in the centre of the heel with subjects in a supine and semi-weight bearing positions. The present technical paper describes materials, construction, calibration, recording procedure, advantages and limitations of the system with a view of future developments.

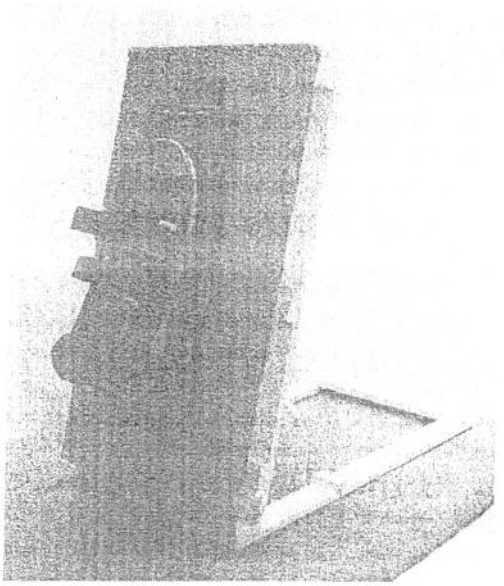

Fig. 1. Device in the supine position.

2. Methods

2.1. System development

Two systems, the laser Doppler fluxmeter (refer footnote 3) and a pressure sensor, were integrated. The laser Doppler fluxmeter was chosen since it can measure skin blood flow non-invasively. It records mean blood cell flux, number or concentration of moving blood cells, mean speed of blood cells over a period of time and skin temperature. In order to measure the amount of pressure applied to the skin, a pressure sensor type LM-50KA (refer footnote 4) was used. The pressure sensor is 20±0.1 mm in diameter and was placed in the piston below the laser Doppler fluxmeter probe. As pressure was applied, the sensor was able to quantify the pressure applied by the piston onto the skin (see Figs. 1 and 2).

The piston pressure mechanism housed a three-tier piston. The top section, a hollow housing 20 mm × 38 mm high with a groove on one side for the sensor cable to exit, housed a laser Doppler fluxmeter sensor type DP1T/7-14 6 mm × 34 mm high. The middle tier was composed of a strain gauge type LM50KA (refer footnote 4). A 20 mm × 61 mm high bar composed the bottom tier. The piston was housed in a cylinder with a nut and plate welded to one end. A spindle mechanism was fitted to one end of the cylinder. By turning the handle on the spindle mechanism clockwise (or anti-clockwise), the piston applies (or removes) plantar foot pressure to the centre of the heel.

A medical-surgical shoe[5] was modified to remove the rocker-bottom by adhering a layer of ethyl vinyl acetate. The shoe was fitted to a wooden board and the three-tier piston was fitted to the underside with the piston piercing the centre of the heel in the shoe. The medico-surgical shoe was chosen because the toe box is open and can accommodate either foot (left or right) with any associated metatarsophalangeal joint deformity.

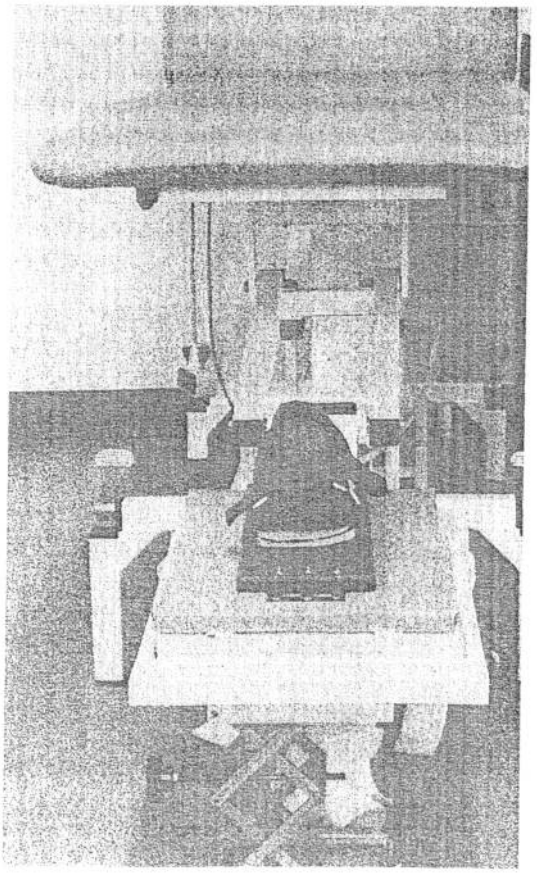

Fig. 2. Device in the semi-weight bearing position.

[2] Applied Laser Technology, Maarheeze, the Netherlands.
[3] Moor Instruments Ltd., UK.
[4] Kyowa Electronic Instruments Co., Ltd., Tokyo, Japan.
[5] Darco International Inc., USA.

A frame was designed to meet the set criteria that the device must be capable of taking recordings with the subject in a supine position and sitting with the legs in dependency position. The frame was fixed with hinges to the underneath of the wooden board and allowed the shoe to be positioned at the end of the couch almost in a vertical position for supine measurements (see Fig. 1). By collapsing the frame, the wooden board could be fitted on a stand allowing the shoe to be parallel with the ground for measurements with the subject in semi-weight bearing (see Fig. 2).

2.2. Calibration

The laser Doppler probes were calibrated using Brownian motion of microspheres in flux standard (refer footnote 3) at a temperature of 20–22 °C. Calibration of the pressure sensor involved placing a calibration vice over the shoe device and using various weights to calibrate the sensor output (see Fig. 3). The pressure sensor produced a linear relationship between applied pressure and sensor output (Pearson's correlation = 1, $P < 0.01$). The average pressure was calculated as the load (force) divided by the piston surface area.

2.3. Subjects

Ethical approval was obtained from Queen Margaret University College. A total of four volunteers (two male and two female) participated in the study with an age distribution of 28 ± 8.6 years. Prior to the study subjects were asked to refrain from alcohol for 24 h, caffeine for 6 h, food for 2 h and not exercise for 1 h prior to the study [14,16]. Subjects taking vasoactive agents were excluded from the study. To achieve a stable recording environment the room was draft-free and the temperature and humidity were kept at 21.7 ± 0.8 °C and $40 \pm 3.4\%$, respectively, as cutaneous blood flow is sensitive to ambient and skin temperature. Volunteers were asked t remove heavy clothing and were allowed to acclimatise fo 20 min to reach a stable baseline condition [17]. Due to re gional variations of cutaneous blood, one site, the heel wa chosen. All subjects were asked not to take deep inspiration or breath quickly, remain immobile and not to talk durin the data collection.

Subjects were randomised into a supine position followe by a semi-weight bearing position or a semi-weight bearin position followed by a supine position. The subjects accli matised in the randomly assigned starting position.

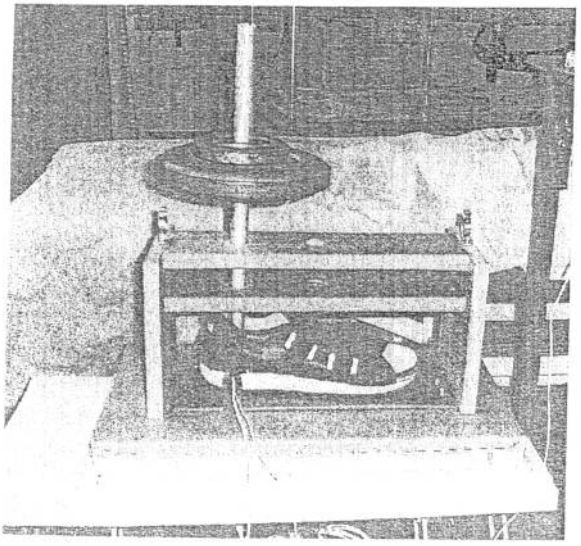

Fig. 3. The calibration vice used to calibrate the pressure sensor with weights.

2.4. Recording procedure

To achieve a reproducible system the foot was placed in the shoe ensuring the heel was in contact with the heel counter and the midline of the third digit was aligned with a mark on the shoe prior to fixing the foot with the shoe straps.

2.4.1. Supine recording procedure

The shoe device was placed in the supine position (see Fig. 1). The randomised right or left foot was then placed in the shoe at zero piston pressure, skin temperature was checked and baseline recording taken for 2 min. A randomised pressure of 20, 40 and 80 kPa was then applied, by turning the spindle, and cutaneous blood flow recorded for 3 min for each pressure value. After the pressure was removed cutaneous blood flow was recorded for 5 min as the signal returned to reference baseline.

2.4.2. Semi-weight bearing recording procedure

The shoe device was placed in the semi-weight bearing frame (see Fig. 2). The recording procedure described before was repeated.

2.5. Data analysis

Data analysis compared supine against semi-weight bearing and was divided into two parts: response to loading and the hyperaemic response post release of pressure. The hyperaemic response post-pressure application may suggest a registration of insult to the soft tissues (Figs. 4 and 5). Other studies have reported findings by interpreting the hyperaemic response [14,17–19] All laser Doppler flowmetry (LDF) readings are in arbitrary units (AU). The baseline LDF was recorded for 2 min prior to the application of pressure in the supine and semi-weight bearing positions. A typical example of an LDF registration for a baseline recording on the plantar aspect of the heel is shown in Fig. 6 for the unloaded period prior to the application of pressure. The flux signal produced by the laser Doppler was constantly fluctuating as a result of the cardiac rhythm, the speed and concentration of blood cells, and other random fluctuations.

All data was referenced to biological zero (BZ), with BZ being the residual noise offset of LDF in the absence of blood and is higher than instrument zero [17]. That is, BZ is

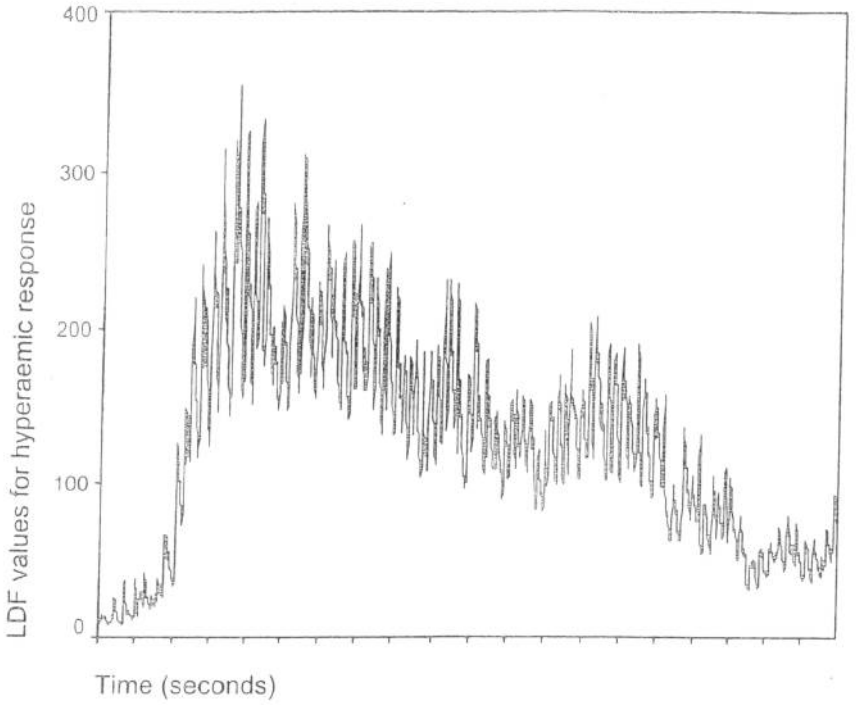

Fig. 4. An example of the hyperaemic response LDF signal following the release of 3 min of pressure on the plantar aspect of the heel.

the value obtained at a particular anatomical site following occlusion of the vascular supply to that area and is higher than the instrument zero. Instrument zero is the value obtained when the laser Doppler probe is held against a white surface [17]. The BZ was considered to be the same as the minimal LDF reading during maximal pressure loading for each subject [14].

2.5.1. LDF levels during pressure loading

The LDF levels during pressure loading (A in Fig. 5) is the decrease in flux following the application of external pressure for a total period of 3 min.

2.5.2. Hyperaemic response

The hyperaemic response was divided into three parts:

- Maximum peak hyperaemic response (B in Fig. 5)—the maximum rise of LDF following release of pressure.
- Peak hyperaemic response relative to baseline (C in Fig. 5)—the maximum rise of LDF following release of pressure from the baseline reading.
- Time to peak hyperaemic response in (D in Fig. 5)—time taken from release of pressure to the highest LDF for the hyperaemic response.

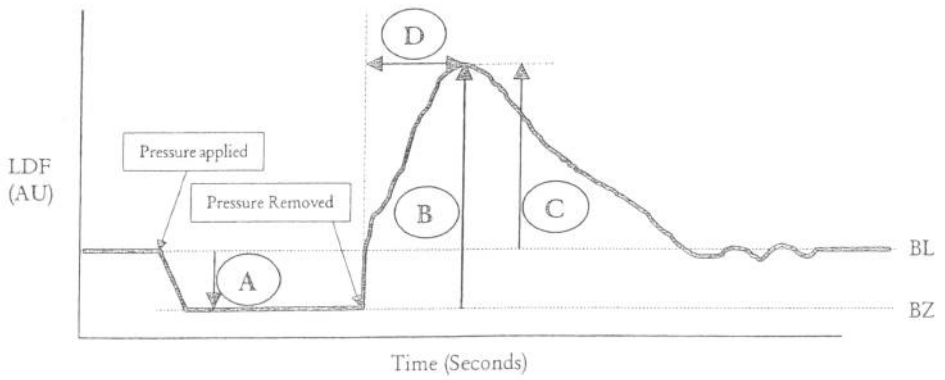

Fig. 5. The analysis of the LDF curve is described. LDF refers to laser Doppler flux measured in AU; BL, baseline LDF; BZ, biological zero of LDF; A, the decrease in LDF following the application of pressure; B, the maximum peak LDF attained from BZ following release of pressure; C, the absolute peak LDF attained from BL following release of pressure; and D, the time taken in seconds to reach the highest LDF.

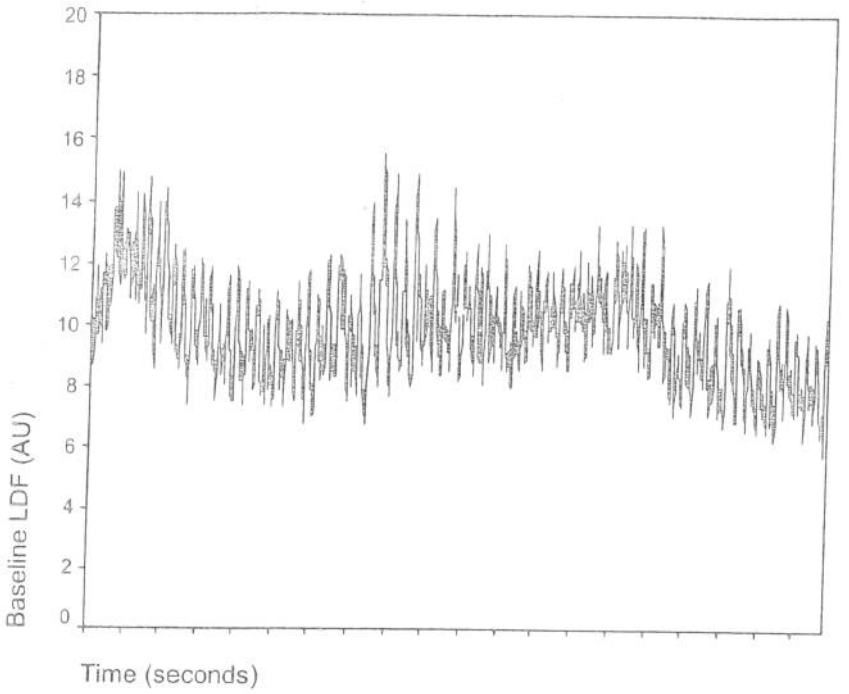

Fig. 6. An example of a baseline LDF signal on the plantar aspect of the heel.

3. Results

3.1. LDF levels during pressure loading

During pressure loading there was a reduction in all LDF values (see Figs. 7–10). The reduction in the total LDF levels following the application of pressure, as shown in Table 1, was greater in semi-weight bearing position compared to the supine position. Wilcoxon's paired sample test showed a significant difference between blood flow reductions following the application of pressure ($P = 0.015$) in the supine and semi-weight bearing positions. There was no significant

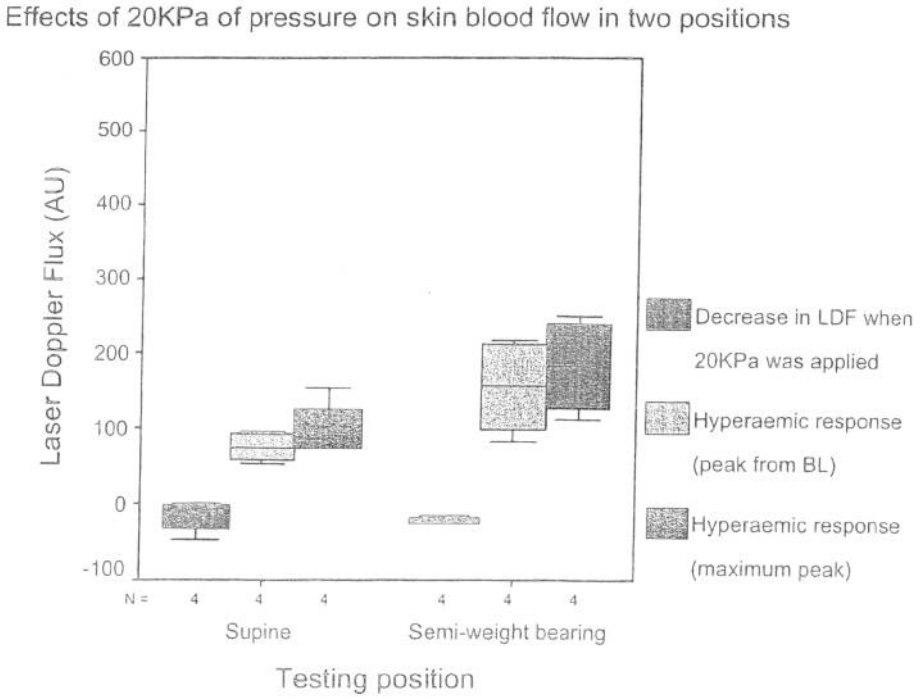

Fig. 7. The effects of applying 20 kPa of pressure to the skin for 3 min and the resultant hyperaemic response.

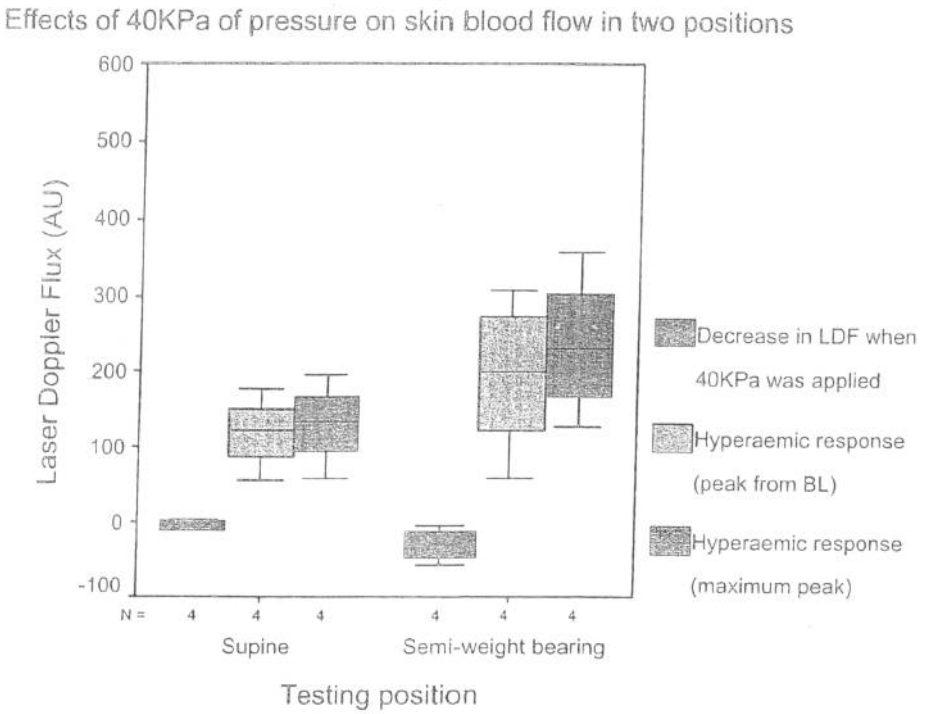

Fig. 8. The effects of applying 40 kPa of pressure to the skin for 3 min and the resultant hyperaemic response.

increase or decrease in probe/skin temperature between baseline and pressure applied for both positions.

3.2. Hyperaemic response

The hyperaemic response occurred following the release of pressure applied after a total duration of 3 min. Fig. 6 shows a typical hyperaemic response (the fluctuating flux signal is characteristic of the laser Doppler and has been explained earlier). Note that immediately after the release of applied pressure there is a surge of blood flow resulting in the maximum peak LDF. Following the maximum peak LDF, there is a recovery phase as the LDF settles back to baseline. The maximum hyperaemic response (see

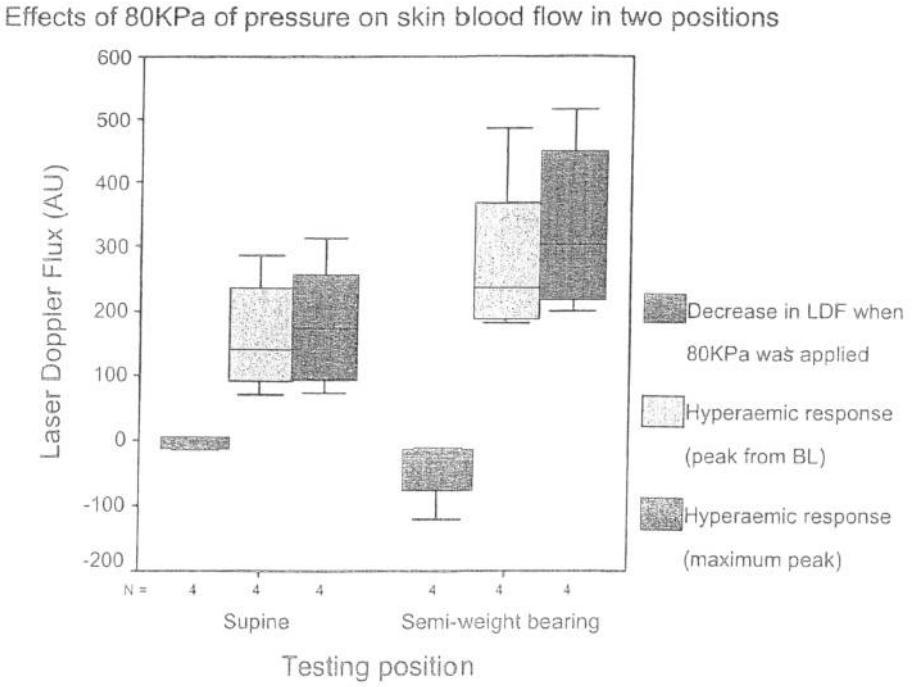

Fig. 9. The effects of applying 80 kPa of pressure to the skin for 3 min and the resultant hyperaemic response.

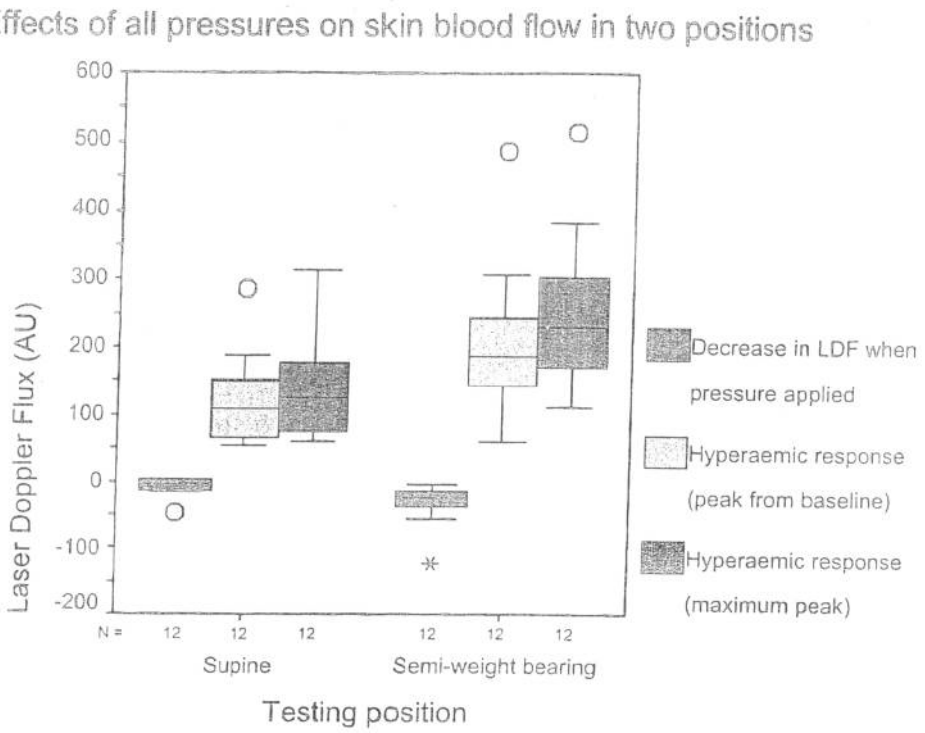

Fig. 10. The effects of applying all the various pressures to the skin for 3 min and the resultant hyperaemic response.

Table 1
Effects of application of pressure on the microcirculation of the skin in the heel

Applied pressure (kPa)	Position	Pressure applied decrease in LDF from baseline	Hyperaemic response		
			Time to peak	Maximum peak	Peak from BL
20	Supine	−8.8 (47.2)	11.9 (64.8)	85.5 (79.1)	77.4 (42.1)
	Semi-weight bearing	−20.6 (6.3)	42.5 (126.4)	185.9 (137.7)	157.0 (134.8)
40	Supine	−3.6 (13.7)	5.2 (195.6)	133.9 (137.7)	121.4 (121.5)
	Semi-weight bearing	−31.1 (52.1)	61.8 (3.7)	230.6 (227.6)	209.1 (247.4)
80	Supine	−2.1 (19.8)	5.0 (36.6)	158.7 (241.7)	149.6 (216.5)
	Semi-weight bearing	−25.1 (109.9)	60.1 (59.2)	307.5 (314.4)	221.1 (306.3)
Total median	Supine	−4.9 (50.3)	5.2 (197.5)	124.2 (253.9)	104.2 (231.3)
	Semi-weight bearing	−22.4 (117.1)	57.7 (137.4)	230.2 (230.2)	197.35 (426.9)
Wilcoxon's paired samples test between supine and semi-weight positions		$P = 0.015$	$P = 0.034$	$P = 0.006$	$P = 0.028$

The median values (range) for the application of various levels of pressure in the supine and semi-weight bearing positions. All LDF skin blood flow values are in arbitrary units, except for the "Time to peak" which is in seconds.

Table 1 and Figs. 7–10), was significantly higher in the semi-weight bearing position than the supine position ($P = 0.006$). Similarly, the peak response from baseline was elevated in the semi-weight bearing position compared to the supine position ($P = 0.028$) (see Table 1 and Figs. 7–10). The time taken to reach the maximum peak hyperaemic response was also greater in the semi-weight bearing position than the supine position ($P = 0.034$) (see Table 1 and Figs. 7–10).

4. Discussion

The study describes the development of a system to measure the effects of plantar foot pressure on skin blood flow in various positions (i.e. supine and semi-weight bearing). The paper focuses on design materials, construction, calibration, recording procedure, data analysis method and the limitations of the current equipment. The synchronised equipment allows for the measurement of the effects of quantifiable

externally applied pressure on cutaneous blood flow of the plantar aspect of the heel in a supine and semi-weight bearing position. It is also possible to adapt the current equipment to measure the effects of static standing plantar foot pressure on skin blood flow in the plantar aspect of the centre of the heel.

The laser Doppler fluxmeter (refer footnote 3) provides non-invasive, real time measurements of local tissue blood flow. The instrument works on the principle of Doppler shift of laser light, which penetrates to approximately 1 mm and is reflected by passing objects. For example, when laser light is reflected off a moving object (blood cell), its frequency is shifted and the amount of shift is dependant on the speed of the moving object.

When carrying out experiments into the effects of pressure on cutaneous blood flow it is important to reduce variables that might affect the reliability and validity of results. Movement artifacts, deep inspirations or talking will affect the quality of the LDF signal and hence it is important for subjects to comply with instructions while data is recorded. One limitation of the current system is that probe/skin movement produces a signal disturbance, as could be observed during application of pressure, which stopped when the piston remain stationary. As a result the current system is unsuitable to be used for measuring the effects of plantar foot pressure on skin blood flow during walking.

Bircher et al. [17] and Ingolfsson et al. [20] reported on how the quality of the LDF signal is also dependent on environmental experimental conditions and probe positioning. In addition, Engelhart and Kristensen [21] compared the laser Doppler fluxmeter against the ^{133}Xenon technique to measure cutaneous blood flow and concluded that the laser Doppler fluxmeter not only measures capillary blood flow but also blood flow in the arteriovenous anastomoses which is part of the temperature regulation mechanism. Thus, it is vital to control the environmental temperature to reduce the thermoregulatory mechanisms affecting the results. Probe positioning is also vital for repeatability and reproducibility of results [17,20]. The advantage of the developed system is that the laser Doppler fluxmeter and pressure probes are held in one position within the piston mechanism. In addition, to ensure that the same area on the plantar aspect of the heel was sampled, guide lines where drawn on the shoe to allow correct alignment of the foot. Following alignment, the foot was strapped to ensure that no foot displacement occurred from the correctly aligned position.

On standing, as a result of gravity, postural vasoconstriction occurs to prevent the formation of oedema in the lower limb. This physiological mechanism limits the increment in capillary pressure, reduces blood flow and allows intracapillary osmotic pressure to rise and oppose the continued transcapillary filtration of fluid in the dependant leg [22]. Significant differences were found between baseline blood flow with the subject in a supine and semi-weight bearing positions. Following the application of pressure the decrease in LDF values from baseline was greater in the semi-weight bearing position when compared to the supine position and were statistically significant. The greater decrease in total LDF semi-weight bearing values suggests that applying plantar foot pressure to a vascular bed, in the plantar aspect of the heel, which is already subject to postural vasoconstriction seems to cause a greater reduction in cutaneous blood flow.

Previous studies have shown a difference between LDF values in the supine position and in dependency in areas with and without arteriovenous shunts [22,23]. In addition, in areas of skin where arteriovenous shunts are numerous, the reduction in cutaneous blood flow on dependency was abolished by the release of sympathetic tone [22]. Since the laser Doppler fluxmeter partly measures arteriovenous shunts and the soles of the feet are rich in arteriovenous shunts, our results are partly affected by this and do not solely record nutritional blood flow. This study's reactive hyperaemic responses reflect both, the nutritive blood flow and effects on arterioveneous shunts following pressure ischaemia.

Reactive hyperaemia is a manifestation of the repayment local blood flow regulation mechanism that is set into motion after a period of vascular occlusion and lasts long enough to repay the tissue oxygen and nutrient deficit that accrued during the vascular occlusion period. The mechanism emphasises the close relationship between local blood flow regulation and supply of nutrients to the tissues [21,24]. Thus, it may reflect the level of insult to the soft tissues with the greater the insult the greater and longer the hyperaemic phase and vice versa. The study suggests that differences exist between measuring the hyperaemic response following pressure ischaemia in a supine and semi-weight bearing position (see Table 1). It may be that gravity may be related in producing a quicker and larger hyperaemic response in the foot when the subject is upright as compared to the subject in a supine position where the effects of gravity on blood flow to the foot is minimised.

5. Conclusion

This paper focuses on the design and development of a device to measure the effects of quantifiable plantar foot pressure on skin blood flow in the centre of the heel with subjects in a supine and semi-weight bearing. Although further research is needed to answer some of the questions arising from this study, the physiological response to plantar foot pressure acting on cutaneous blood flow differs if measured in a supine or semi-weight bearing position. Thus, it may be more important to measure physiological cutaneous blood flow responses to plantar foot pressure with the subjects in an upright position if we are to understand what occurs during ambulation and its relationship to tissue viability.

The device has been improved by reducing the piston surface area, adding a capacitive pressure sensor (refer footnote 1) and synchronising the DRT4 laser Doppler fluxmeter (refer footnote 3) with the pressure sensor. The improved device

is currently being used to compare the effects of plantar foot pressure on skin blood flow between controls and subjects with rheumatoid arthritis or diabetes mellitus.

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22.2 A review of the effects of plantar foot pressure on skin blood flow.

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Review

A review of the effects of external pressure on skin blood flow

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Abstract

The human foot is a complex mechanical structure consisting of bones, ligaments and joints. They act together to provide a robust system capable of absorbing and dissipating the intermitted pressure that is subjected to its plantar surface during walking to prevent soft tissue breakdown. Current studies suggest that plantar foot pressure may lead to soft tissue breakdown (e.g. neuropathic ulceration) and hence research has so far concentrated on investigating the mechanical effects of plantar foot pressure on the foot's integrity. This has been possible through the widely available pressure and force platforms as well as in-shoe pressure systems. However, to understand how plantar foot pressure causes soft tissue breakdown it is vital to investigate both the physiological–mechanical interactions between the skin and plantar foot pressure. This review suggests that with the current advances in technology, the physiological response of skin blood flow to mechanical plantar foot pressure should be investigated and correlated further, both during static and dynamic loading, by developing a new system capable of either measuring both variables simultaneously or by synchronising two systems in real time.

Keywords: Plantar foot pressure; Skin blood flow; Laser Doppler fluxmeter

1. Introduction

During human walking the tissues in the plantar aspect of the foot are subjected to intermittent pressure. Evolution has adapted the soft tissues in the plantar aspect of the foot to cope with these "normal" insults to the skin's integrity. However, some studies have shown that in cases where peripheral neuropathy is present, high plantar foot pressure may lead to soft tissue breakdown and ulceration. As a result, over the years, research has concentrated on the effects of plantar foot pressure on foot function and integrity. Probably, because reliable and practical equipment has been readily available for quite a number of years. Indeed many devices exist that allows the study of plantar foot pressure from the early Harris and Beath mat to the more modern platforms and in-shoe computerised systems. However, little is known of how plantar foot pressure interacts or causes damage to soft tissues. To understand how plantar foot pressure causes soft tissue breakdown it is vital to investigate the physiological–mechanical interactions between the skin and plantar foot pressure. With currently available technology, although in its infancy, it may now be possible to investigate the effects of plantar foot pressure on skin blood flow. This review concentrates on studies to date who have developed prototypes of such systems.

2. Overview of devices to measure the effects of external pressure on skin blood flow

Various methods of investigating the effects of external pressure on the skin's microcirculation have been developed by a number of authors. However, most of the studies do not relate to the foot [1–7,19].

Yamaguchi et al. [1] developed a system to measure the effect of orthodontic brace forces on the peripheral circulation of the upper border of the gingival in the mouth using a laser Doppler fluxmeter (LDF) fiber-optic probe fixed to a plate to hold the probe at 90° to the gingival. Pressure was applied to the teeth using springs that were hooked to buccal tubes bonded to the maxillary central incisors.

Holloway et al. [2] developed a system to measure the effects of external pressure loading on the microcirculation

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of the skin in the volar aspect of the forearm using ^{133}Xe clearance. The system involved injecting 30 μCi ^{133}Xe dissolved in 0.02 ml of sterile, pyrogen-free physiological saline into the skin using a 30-gauge needle and Hamilton syringe. This was followed by applying external pressure, using a servo-controlled loading device with an overall system frequency of 0.5 Hz, which applied the load on a flat Plexiglas disc of 3 cm diameter centered on the site of injection.

Karlsmark and Kristensen [3] designed a device to measure the effects of pressure-relieving materials in the prevention of pressure ulcers in the sacral area. The device allowed for measurement of pressure on the sacral region when subjects lie in a supine position by placing a LDF probe (Perimed, Sweden) in small-bore holes in the polyacrylate board just below the central part of the sacral area. Following baseline recording, a circular closed cell foam plate (Comfeel PRD) with the centre removed was positioned under the sacral region. Through the hole, the LDF probe was placed for the second recording of cutaneous blood flow in the sacral region. Similarly, Schubert and Héraud [4] developed a system to measure sacral skin microcirculation in elderly stroke patients in a supine and semi-recumbent position. The design consisted of an LDF probe (Perimed), fixed to the skin on the sacral area using an angle-probe holder, which was embedded in a specially designed foam rubber pad covered with terry cloth to ensure that the probe did not influence skin pressure. Colin and Saumet [5] further developed a different device to measure skin blood flow and transcutaneous oxygen tension ($tcpO_2$) on the sacral area. This device consisted of an easily adjustable, height and length, mobile arm. At the end of the arm, pressure on the probe holder was controlled by the amount of compressed air in the air chamber. Below the air chamber a strain gauge (Scaime, Annemasse, France) measured the pressure applied over the sacral area. The probe holder, below the strain gauge, made skin contact with the sacral area and contained both the LDF probe (Perimed) and the $tcpO_2$ (Kontron Instruments, Watford, UK) probes. In another study, Schubert and Fagrell [6] developed a device to measure the effects of pressure on skin blood flow on the sacral and Gluteus Maximus area. The device consisted of a pivoted arm with a plexiglass head with a pressure cub containing an LDF probe fitted through a hole in the center. The plexiglass head with a pressure cub was counter-balanced with weight on the opposite site of the arm. Pressure was applied on the head by a sliding weight and skin blood flow was recorded using the LDF. Pressure was recorded using a thin pressure sensor (Kyowa Electronic Instruments Co., Ltd., Tokyo, Japan) and temperature with a thermistor probe connected to a digital display (MC 8700, Exacon, Copenhagen, Denmark). Both probes were located under the plexiglass bottom plate.

Fromy et al. [7] developed a device to apply progressive calibrated pressure to the skin of the finger. The device is similar to that developed by Abu-Own et al. [8] in that it uses a pivoted arm. With the subject in a supine position the laser Doppler probe (PF408, Periflux; Perimed, Järfälla, Sweden) is attached to one end of the pivoting arm. Next to the laser Doppler probe a plastic container is attached with one end of a polyethylene capillary tube secured in the container and the other to a syringe filled with water in an automated syringe pump. Weights are attached to the other end of the pivoted arm to counter-balance the laser Doppler probe on the opposite site. With the subject in a supine position and the finger resting on a block with the laser Doppler probe counter-balanced and just making skin contact, the automated syringe pump is started. As the plastic container fills with water progressive pressure is applied to the skin.

Studies investigating the effects of plantar foot pressure on skin blood flow are few but are leading the way towards the development of such equipment [8,9,12,13,16–18,20]. All these studies may be classified according to the type of equipment used to measure skin blood flow, which includes fluorescein flowmetry, laser Doppler imaging, and laser Doppler fluxmetry.

3. Studies using fluorescein flowmetry

Fluorescein flowmetry is an invasive method requiring the subject to drink 15 ml of 50% alcohol followed by a bolus injection of 7 mg of sodium fluorescein per kilogram of body weight in a 10 ml of isotonic saline injected into a cubital vein. Images are then taken of the subject's soles and blood flow is expressed as an index between the maximum fluorescence obtained between the first passage of sodium fluorescein through the circulation and the rise time. This is defined as the first interval of time between 10 and 90% of maximum fluorescein [9,10].

Proano et al. [9] developed a system to measure plantar foot pressures using one of the EMED Gait Analysis Systems (Novel GmbH, Munchen, Germany) followed by measuring supine, standing and walking skin blood flow using fluorescein flowmetry. The standing and walking fluorescein flowmetry were carried out with the subjects standing on a podoscope and pictures taken using a Hasselblad electric motor-driven camera 500 EL/M (Hasselblad, Gothenburg, Sweden). However, one of the limitations of the study was that plantar foot pressures and plantar circulation were not measured simultaneously. Also, during the walking measurements the subjects were trained to stop on the podoscope and lifting the opposite foot for some of the pictures to be taken, leading to a one legged standing position rather than a true walking position. Finally, this method uses invasive procedures requiring the subject to drink 15 ml of 50% alcohol followed by a bolus injection of 7 mg of sodium fluorescein per kg of body weight in a 10 ml of isotonic saline injected into a cubital vein. Thus, one may question the practicalities and ethics of such procedure, especially if one wants to study a high-risk group.

4. Studies using laser Doppler imaging

Laser Doppler imaging is a non-invasive method to measure skin blood flow with the device making no skin contact and is ideal to measure skin blood flow on skin grafts and ulcers. The system works on the principle of laser Doppler wave shift principle. The low power laser is used to scan an area on the skin and is controlled by an optical scanner consisting of two mirrors. At each measurement the laser penetrates to a depth of a few hundred micrometers and reflected back to a photo-sensor positioned in the scanner head. The laser light, where it interacts with moving cells, is spectrally broadened (termed the Doppler effect). This is converted into an electrical signal and stored in the computer as a product of blood cell speed and concentration (i.e. flux). During each measurement procedure the captured perfusion value is colour coded. At the end of a scan a colour coded image representing blood flow is shown [11]. However a scan of an area 12 cm × 12 cm with a maximum of 4096 measurement sites takes approximately 4.5 min [11], thus its application in dynamic ambulation is questionable.

Mayrovitz et al. [12] using the laser Doppler imaging (LDI) system (LISCA Development AB, Linköping, Sweden) was able to measure heel blood perfusion responses to pressure loading and unloading in women. Subjects were placed in a supine position with their feet extended over the edge of the examination table. A baseline LDI scan of the plantar aspect of both heels was carried out at a distance of 19 cm. External pressure was applied to the right heel, for a period of 40 min, using a clear plastic plate 3 mm thick and a second scan taken (a limitation of this study is that the externally applied pressure was not quantified and may have been different between subjects). During this period scans were taken of the off-loaded left heel. After the right foot was off-loaded scans were taken of the heel. At the end of each experiment scans of background calibration white card (Gray Card, Eastman Kodak Company, Rochester, New York) were taken with and without the plastic plate interposed to offset the calibration value which was subtracted from the raw data collected to account for any effects associated with light transmission through the plastic plate. Mayrovitz and Smith [13] used the same technique developed by Mayrovitz et al. [12] (already explained above) to study heel skin microvascular blood perfusion responses to sustained pressure loading and unloading in the supine position.

5. Studies using laser Doppler flowmetry

Laser Doppler flowmetry provides non-invasive, real time measurements of local tissue blood flow. The instrument works on the principle of Doppler shift of laser light by passing objects. That is, when laser light is reflected off a moving object (blood cell), its frequency is shifted and the amount of shift is dependent on the speed of the moving object. The system consists of a solid-state laser emitting diode of 780 nm and a receiving photo-detector. A computer is used to collect and analyse the data. From the laser Doppler wave shift the mean blood cell flux, number or concentration of moving blood cells, the mean speed of the moving blood cells and the detected total backscatter light can be worked out [14–17]. A temperature sensor may be added to the laser probe to calculate skin temperature.

Abu-Own et al. [8] developed a method to measure the effects of quantifiable external pressure on the posterior aspect of the calcaneus with the subject in a supine position using a pivoted arm mounted on a stand and the Perimed PF 2B laser Doppler flowmeter (Perimed). On one end of the pivoted arm a 5 cm diameter pressure-applying acrylic indenter housed the low profile laser Doppler probe and an interface pressure sensor (Talley Group Ltd., Romsey, Hants, UK). With the laser Doppler probe making skin contact forces of 50–1500 g were applied to the skin on the heel region, using a cantilever mechanism with a central pivot, and loading weights on the opposite end of the arm. The device was designed to measure the effects of compression on the heel's microcirculation in different hospital beds. In another paper, Abu-Own et al. [18] developed a system to measure the effects of intermittent pneumatic compression of the foot on the microcirculatory function in arterial disease. The device consisted of an impulse foot pump (A-V Impulse System, Novamedix Ltd., UK), used to activate the foot muscle pump, which was fitted to a purpose-made slipper and made contact with the plantar aspect of the foot. Inflation and deflation applied intermittent compression to the foot. A LDF probe and a $tcpO_2$ probe, attached to the pulp of the big toe and dorsum of the foot respectively, measured the skin's microcirculatory function and transcutaneous oxygen tension. The device was unable to measure the direct effects of external pressure on the microcirculation of the foot.

Abu-Own et al. [19] used a LDF probe, fitted in a polyethylene chamber and applied to the supramalleolar region underneath a blood pressure cuff, to measure the effects of leg compression in patients with chronic venous insufficiency. The LDF probe was fitted 8 cm above the medial malleolus. The polyethylene chamber and the blood pressure cuff were connected together via a Y junction to ensure that the reading on the sphygmomanometer reflected the pressure being exerted on the skin below the polyethylene chamber.

Meinders et al. [20] developed a device to measure the microcirculation of the center of the plantar aspect of the heel under quantifiable external pressure with the subject in a supine position. A shoe with a hole in the centre of the heel was attached to a rigid board at the end of the bed. A screw piston with a load cell (HBM load cell, type Z8) 2 cm in diameter and a LDF probe (Applied Laser Technology, Maarheeze, The Netherlands) was inserted into the hole in the heel of the shoe. The subject's foot was placed in the shoe with the LDF probe making skin contact. Quantifiable external pressure was applied by turning the piston. Recording of pressure and blood perfusion was carried out using

a computer. However, this system was only visually synchronised and only capable of taking supine measurements. Since human walking involves an upright posture and differences in skin blood flow exist between the patient in a supine and upright positions, due to the veno-arterial response causing vascular constriction when standing, the value of taking supine plantar foot pressure measurements is very limited.

Cobb and Claremont [16] developed a dynamic in-shoe laser Doppler fluxmetry system to measure the effects of plantar foot pressure on skin blood flow over the metatarsal head area in diabetics. The system consisted of a laser Doppler sensor incorporated into a shoe with a pressure sensor. The pressure sensor was used as a pressure switch, enabling analysis of the loaded and unloaded foot. A mobile recording box enabled data collection without the use of trailing cables, with data downloaded to a computer at a later time for analysis. Although plantar foot pressure was not quantified, results showed that following loading an almost linear increase in skin perfusion blood flux occurred during the swing phase. The short time phase of loading in normal subjects was not sufficient to induce a hyperaemic response. However, in periods of prolonged weight bearing (e.g. when standing for 2 min) followed by non-weight bearing, a hyperaemic response was induced [16].

Santos et al. [17] developed a system, similar to Meinders et al. [20], using a pressure sensor type LM-50KA (Kyowa Electronic Instruments Co., Ltd.) capable of measuring the effects of quantifiable plantar foot pressure on skin blood flow in the centre of the heel in an upright position using an integrated pressure-blood flow piston mechanism housed in a medical-surgical shoe (Darco International Inc., USA). The system has now been modified to use two widely available systems allowing for minimum equipment development and accessibility of this type of equipment for future research. The laser Doppler fluxmeter DRT4 (Moor Instruments Ltd., UK) has been integrated with the Pedar standard and mobile (Novel GmbH) that uses a pressure capacitive sensor. The devices are electronically synchronised in real time using a specially developed midway synchronisation box. The advantages of this system are that both quantifiable plantar foot pressure and duration of pressure application can be controlled when investigating the effects of plantar foot pressure on skin blood flow in an upright position. However, this equipment is not capable of measuring the effects of dynamic plantar foot pressure on skin blood flow.

6. Conclusion

Research in the foot has mainly concentrated on plantar foot pressure, with high plantar foot pressure correlated with neuropathic ulceration. These studies have suggested a mechanical interaction between plantar foot pressure and tissue viability, however the mechano-physiological processes may not be investigated using pressure systems alone. This review suggests that with current technology the physiological responses of skin blood flow to mechanical plantar foot pressure should be investigated in more detail, both during static and dynamic loading, and more work needs to be done to perfect these techniques and make such equipment widely available.

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